

MORPHOLOGICAL AND MOLECULAR ANALYSIS OF THE ANDEAN SLUGS
COLOSIUS CONFUSUS, N. SP., A NEWLY RECOGNIZED PEST OF CULTIVATED
FLOWERS AND COFFEE FROM COLOMBIA, ECUADOR AND PERU, AND
COLOSIUS PULCHER (COLOSI, 1921) (GASTROPODA, VERONICELLIDAE)

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ABSTRACT

In this study, we propose *C. confusus*, new species, an Andean slug of the genus *Colosius* Thomé, 1975, and a newly recognized pest of coffee and cultivated flowers from Colombia, Ecuador and Peru. We compare it with *C. pulcher* (Colosi, 1921), a poorly known species with which it has been confused. Our study is based on morphological analysis of a large number of specimens, including interceptions on cut-flowers and live plants by federal agricultural inspectors of the United States Department of Agriculture (USDA) and the Department of Homeland Security (DHS), and material from eight museum collections. Genetic diversity within *C. confusus*, n. sp. and *C. pulcher* is also analysed based on fragments of cytochrome oxidase I (COI), and 16S rRNA. They are differentiated by reproductive characters and genes studied. In *C. confusus*, n. sp., the phallus has a deep longitudinal groove from the base, near the retractor muscle, to its distal region, close to the papilla. In *C. pulcher*, there is an oval to rectangular swelling on the basal region of the phallus. Some important differences between these species are also found in the digitiform gland and bursa copulatrix. We describe, illustrate and discuss the color variation, morphological similarities, diagnostic characters and its variation, habitat and distribution for each species. Genetic diversity within *C. confusus*, n. sp., and *C. pulcher* is low. In order to analyze their relationship with *C. propinquus* (Colosi, 1921) (currently a junior synonym of *C. pulcher*) and *C. lugubris* (Colosi, 1921) (type-species of *Colosius*), fragments of COI, 16S, and 28S rRNA genes are also analyzed in a sample of these species. *Colosius confusus*, n. sp., is a distinct lineage within the genus *Colosius*. It is not a sister species of *C. pulcher*, which has *C. propinquus* as a sister species, here recognized as valid. *Colosius confusus*, n. sp., is closer to the clade that includes *C. pulcher* and *C. propinquus* than it is to *C. lugubris*. Based on the phylogenetic reconstruction, *C. lugubris* is sister to all the other *Colosius*, although additional studies are required to formally test phylogenetic placements and monophyly of the genus. Associated imports and number of interceptions per year of *C. confusus*, n. sp., by agricultural inspectors are also presented.

Key words: land slug, morphology, Ecuador, Peru, Colombia, 16S, COI, 28S.

INTRODUCTION

The slugs of the Veronicellidae Gray, 1840, have been classically distinguished from other pulmonates by the absence of a shell and a developed pulmonary cavity. They are found throughout the tropical and subtropical regions (Thomé, 1993). Some species are agriculturally significant causing serious damage and losses to numerous crops (Andrews & Pilz, 1985; Ca-

ballero et al., 1991; Thomé, 1993; Chiaradia & Milanez, 1999; Chiaradia et al., 2004; Robinson & Hollingsworth, 2005). Some are intermediate hosts of *Angiostrongylus costaricensis* Moreira & Céspedes, 1971, and *Angiostrongylus cantonensis* (Chen, 1935), nematodes responsible for abdominal angiostrongyliasis and eosinophilic meningoencephalitis, respectively, which can accidentally infect humans (Graeff Teixeira et al., 1989, 1994; Rambo, 1997; Laitano et

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al., 2001; Caldeira et al., 2007; Ohlweiler et al., 2010). Despite their agricultural and public health importance, studies of their systematics, distribution and biology are still lacking for most species of the family.

Colosius Thomé, 1975 is one of the eighteen currently recognized genera of the Veronicellidae from the Americas. According to Thomé (1975), it is reported in Ecuador, Peru, Colombia, the Dominican Republic and Haiti and consists of five species: *C. buergeri* (Simroth, 1914) (described from the Dominican Republic), *C. festae* (Colosi, 1921) (described from El Pun, Ecuador), *C. lugubris* (Colosi, 1921) (type-species from Quito, Ecuador), *C. propinquus* (Colosi, 1921) (described from El Pun, Ecuador) and *C. pulcher* (Colosi, 1921) (described from Quito, Ecuador). In the diagnosis provided by Thomé (1975) for the genus, he described as features shared by these species: the rectum penetrating in the tegument close to the female genital pore; the presence of a digitiform gland, with tubules differentiated in two sets, one with short and unbranched tubules and another with long and branched tubules; an oval or globular bursa copulatrix without a “cabecote” (= like a small tip in the bursa proper where the vas deferens penetrates), a short and, in general, thick duct; a short canalis junctor that penetrates in the bursa proper, next to the duct or in the duct, auxiliary to the bursa; and lack of any accessory structure.

In this study our main objective was to describe morphologically a new species of *Colosius*, currently the second most intercepted mollusk species in Miami by federal agricultural inspectors in cut-flowers from Ecuador and Colombia. A secondary objective was to compare it with *C. pulcher*, a species known only from its original description (Colosi, 1921) and re-description of its lectotype (Thomé, 1970b), with which it has been confused. We describe, illustrate and discuss the color variation, morphological similarities, diagnostic characteristics and variation, habitat and distribution for both species. Levels of genetic divergence between species and genetic diversity for them are also analyzed based on the fragments of cytochrome oxidase I (COI) and 16S rRNA genes. Their relationship with *C. propinquus* (Colosi, 1921) (currently regarded as a junior synonym of *C. pulcher*) and *C. lugubris* (Colosi, 1921) (type-species of *Colosius*) are also investigated based on COI, 16S and 28S rRNA. Imports in which specimens were intercepted and number of federal interceptions per year for *C. confusus* are presented.

MATERIALS AND METHODS

Morphological Analysis

A total of 281 specimens intercepted by federal agricultural inspectors were morphologically analyzed. They are deposited in the USDA's National Malacology Laboratory collection at the Academy of Natural Sciences of Drexel University in Philadelphia (Pennsylvania, U.S.A.), and in the USDA Miami Plant Inspection Station collection (Florida, USDA). Eleven lots of *C. confusus* collected in Peru and Ecuador and four lots of *C. pulcher* collected from Ecuador were also analyzed and are deposited in the same collection. Lots from the following institutions were also analyzed: Museo Regionale di Scienze Naturali di Torino (MSNT) (Torino, Italy) (paralectotypes), National Museum of Natural History, Smithsonian Institution (USNM) (Washington, D.C., U.S.A.), Florida Museum of Natural History (FMNH) (Gainesville, Florida, U.S.A.), Zoological Museum University of Copenhagen (ZMUC) (Copenhagen, Denmark, Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ-M) (Quito, Ecuador), Natural History Museum of the National University of San Marcos (MUSM) (Lima, Peru) and Superintendência de Controle de Endemias do Estado de São Paulo (CMS-DPE) (São Paulo, Brazil). Among these specimens, 70 correspond to *C. confusus* (besides 281 specimens from interceptions in August 20th, 2012) and 154 specimens correspond to *C. pulcher*. Two specimens of *C. propinquus* (QCAZ-M-0 03700 and QCAZ-M-003659), and one of *C. lugubris* (USDA-110426) were also analyzed.

In order to analyze the internal morphology, each specimen was dissected under a Nikon® SMZ1500 stereomicroscope. The numbers of tubules of the digitiform gland was determined from 40 randomly selected specimens of *C. confusus*, n. sp., and *C. pulcher*. Digital images of the diagnostic structures were obtained using a Nikon® digital Sight DS-Fi1 camera attached to the Nikon® SMZ1500 stereomicroscope. Multiple images were combined using Helicon Focus 4.03 Pro to increase depth of field, and subsequently histogram normalized using Adobe® Photoshop CS5. Drawings based on these photographs were made using CorelDraw® X5. Morphological characteristics described and illustrated for the species were those considered diagnostic for Veronicellidae (Hoffmann, 1925; Coifmann, 1935; Forcart, 1953; Thomé, 1970a, b, 1972, 1975; Gomes & Thomé, 2001).

Distribution

Records of occurrence, geographical coordinates and altitude were obtained from the lots examined and literature (Colosi, 1921; Hoffmann, 1925; Thomé et al., 1997; Thomé et al., 2001). Coordinates and altitude not obtained by the collectors were found using GoogleMaps. Coordinates were plotted on maps using the program Versamap version 2.07.

Federal Interceptions

Data related to interceptions of *C. confusus*, n. sp., including country of origin, date of interception and type of import in which the specimen was intercepted were obtained from the Agriculture Quarantine Activity Systems (AQAS) of the Plant Protection and Quarantine (PPQ) of the Animal and Plant Health Inspection Service (APHIS), of the United States Department of Agriculture (USDA), based on 281 records of interception obtained as of August 20th, 2012.

Molecular Analysis

From the intercepted and field collected material and recently collected material, a subset was selected for DNA analysis. This included 59 specimens of the new species *C. confusus* from Colombia, Ecuador and Peru and eight specimens of *C. pulcher*, four specimens of *C. lugubris* and two specimens of *Colosius propinquus* from Ecuador, besides six specimens representing outgroups to *Colosius* (Table 1).

DNA was isolated from samples using the DNeasy tissue extraction kit of Qiagen (Valencia, California, U.S.A.). Foot tissue was cut from each specimen using a sterile razor blade and then incubated at 56°C in ATL buffer containing Proteinase K (Qiagen) following the manufacturer's instructions. The foot tissue typically dissolved after one hour of incubation, but incubation times ranged from 1 to 14 hours. DNA was eluted from Qiagen columns using 50–100 µl buffer (Qiagen) and was stored at 4°C until analysis and then at -20°C after analysis. Voucher specimens are deposited in the collection of the USDA's National Malacology Laboratory collection at the Academy of Natural Sciences of Drexel University, in Philadelphia, Pennsylvania, U.S.A., and the Museo de Zoología, Pontificia Universidad Católica del Ecuador, in Quito, Ecuador.

The COI, 16S, and 28S gene fragments were amplified using four separate PCR reac-

tions. The 5' region of the cytochrome oxidase c subunit I (COI) gene commonly used for DNA barcoding of animals was amplified using the primers LCO-1490 (5-GGTCAA-CAAATCATAAAGATATTGG) and HCO-2198 (5-TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al., 1994). The 16 rRNA gene fragment was amplified using 16S-1 (5- CGC-CTGTTTAACAAAAACAT) and 16S-2 (5-ACGC-CGGTTTGAAGCTCAGATC) (Barr et al., 2009). Two separate, non-overlapping fragments were amplified from the 28S rRNA gene. The first 28S fragment included the expansion segment D2. This region was amplified from *C. confusus*, n. sp., (Accession, BX090401-019) using Wade & Mordan's (2000) LSU-2 (5-GGGTTGTTTG-GGAATGCAGC) and LSU-4 (5-GTTAGACTC-CTTGGTCCGTG) primers located in conserved 28S regions C2 and C3, respectively. This primer pair generated multiple bands for several other mollusk samples so alternate primers were designed using an alignment of *C. confusus*, n. sp., *Pedipes mirabilis* (AY465074), and *Vaginulus (Sarasinula) plebeius* (DQ256745) (= *Sarasinula plebeia*). The new primer pair 28S-2F (5-CCGTGAGGGAAAGTTGAAAA) and 28S-4R (5-GTTGCCCAGTCTCTC-CCAGT) is located in C2 and D2 segments, respectively, and amplifies the majority of D2 segment. The fourth fragment amplified the 28S expansion segment D3 using the primers 28S-a (5- GACCCGTCTTGAAACACGGA) and 28S-b (5-TCGGAAGGAACCAGCTAC) located in conserved regions C3 and C4, respectively (Whiting et al., 1997). All four loci (three genes) were used to estimate phylogenetic relationships among species. Additional sequences of 16S and COI were generated for *C. pulcher* (JX629298–JX629311) and *C. confusus*, n. sp., (JX629312–JX629371). 16S locus was used to estimate genetic diversity among *C. confusus*, n. sp., and *C. pulcher* samples.

Reactions were either performed in 50 µl reactions using Ex Taq polymerase (Hot Start version, Takara Bio, Madison, Wisconsin, U.S.A.) or OmniMix HS lyophilized universal PCR reagent beads (Cepheid smart cycler systems, Takara Bio). The OmniMix protocol was used for samples that failed to amplify using the Ex Taq method. Reactions using Ex Taq had final reagent concentrations of 1X buffer, 0.2 mM each dNTP, 0.2 µM each primer, and 0.025 U/µl Taq enzyme. The OmniMix reactions had a final concentration of 0.2 µM of each primer. The Ex Taq and OmniMix protocols used 1 and 2 µl of DNA template, respectively. Water was used as a negative control in all PCR experiments. All

TABLE 1. List of the specimens and outgroups used in the phylogenetic analysis with sampling localities and year of collection, voucher-specimens and GenBank accession numbers.

Taxon	Country	Primary division	Year	Voucher	28S-D2	28S-D3	16S	COI
<i>Sarasinula plebeia</i>	Brazil	Rio Grande do Sul	2001	CMS-DPE 1053	JX532130	JX532142	JX532118	JX532107
<i>Sarasinula linguaeformis</i>	Brazil	Minas Gerais	2006	USDA 120073	JX532131	JX532143	JX532119	JX532108
<i>Semperula wallacei</i>	American Samoa	Tutuila	2005	USDA 110486	JX532132	JX532144	JX532120	JX532109
<i>Laevicaulis natalensis</i>	South Africa	Natal	1995	USDA 10095	JX532133	JX532145	JX532121	JX532110
<i>Phyllocaulis boraceiensis</i>	Brazil	São Paulo	1999	USDA 110485	JX532134	JX532146	JX532122	JX532111
<i>Veronicella cubensis</i>	Antigua	St. Paul Parish	2003	USDA 110484	JX532135	JX532147	JX532123	JX532112
<i>Colosius lugubris</i>	Ecuador	Pichincha	1997	USDA 110426	JX532136	JX532148	JX532124	None
Colosius confusus	Colombia	Intercept	2008	USDA 110483	JX532137	JX532149	JX532125	JX532113
Colosius confusus	Ecuador	Napo	2011	USDA 110422 B	JX532138	JX532150	JX532126	JX532114
<i>Colosius propinquus</i>	Ecuador	Imbabura	1999	QCAZ-M-003659	JX532139	JX532151	JX532127	JX532115
<i>Colosius pulcher</i>	Ecuador	Pichincha	2011	USDA 110419 A	JX532140	JX532152	JX532128	JX532116
<i>Colosius pulcher</i>	Ecuador	Napo	2011	USDA 110421 A	JX532141	JX532153	JX532129	JX532117

reactions were performed on Gene Amp 9700 PCR instruments (Applied Biosystems, Foster City, California, U.S.A.) using the following cycling parameters: 94°C (3 min), 35 cycles of 94°C (20 sec) / 50°C (20 sec) / 72°C (30 sec), and an extension step of 72°C (5 min).

PCR products were visualized on 1% TAE agarose gels stained with ethidium bromide and purified using either the QiaQuick purification kit (Qiagen) or ExoSAP-IT (USB Products, Affymetrix, Cleveland, Ohio) protocol according to the manufacturer’s instructions. All samples were sequenced on an ABI 3730xl DNA Analyzer (GeneWiz Inc., South Plainfield, New Jersey) in both directions using the forward and reverse primers.

The bidirectional trace files generated from the 3730xl were imported into Sequencer v5.0 (Gene Codes, Ann Arbor, Michigan) and edited by hand to generate a consensus file for each sample. The consensus files were imported into MEGA 5 (Tamura et al., 2011) and aligned first using MUSCLE and then adjusted by hand. Alignments are deposited in GenBank and available from the authors. The location of primers and regions of the 28S sequences were identified by comparison to secondary structure of *Apis mellifera* (Gillespie et al., 2006). Regions of ambiguous alignment in the ribosomal gene fragments were recorded and excluded from all subsequent phylogenetic analysis. All sites were used to estimate diversity within a species. The four loci were concatenated into a single alignment for phylogenetic analysis and model testing. Sequences were submitted to GenBank (JX532107–JX532153).

MEGA 5 was used to identify unique genotypes per locus and to estimate pairwise uncorrected genetic distances between *Colosius* specimens and species. Goodness-of-fit to models of evolution for each alignment was tested using corrected Akaike information criterion (AICc, Hurvich & Tsai, 1989) using jModelTest 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) and MEGA5 (Tamura et al., 2011). The jModelTest results selected the transversional model of Posada (2003; TVN + G + I) as the best-fit model and MEGA5 selected the General-Time-Reversible model (GTR + G) as the best-fit model. Based on the similarity of models and compatibility with phylogenetic programs, the GTR + G + I model was selected for subsequent analyses. Maximum Likelihood (ML) trees were generated using PAUP* (Swofford, 2002) and the GTR+G+I model [Lset base (0.2655, 0.1844, 0.2426), rmat (0.5739, 8.7561, 6.8296, 0.8290, 8.7561), shape (0.8570), ncat (4), pinvar (0.5610)].

The ML tree was generated for the concatenated data set using a branch and bound search with no limit on trees saved. An additional heuristic search using 100 bootstrap replicates each of 10 random additions and TBR swapping was performed to estimate branch support. Bayesian Inference (BI) was performed on the concatenated data set using MrBayes 3.1 and the model selected for the ML searches. Tree searches were performed using 1,000,000 generations and sampling every 500 generations. The first 1,000 trees were discarded as a burn-in and four chains were run with a heating parameter of 0.2. Convergence was estimated using the program diagnostic output and the average standard deviations of split frequencies was expected to be less than 0.01. The BI tree was reported as a majority-rule consensus using a 50% cut-off for Posterior Probabilities (PP) for branch support. Additional tree searches were performed on data partitions (16S, 28S-D2, 28S-D3, and COI) to compare with the concatenated tree search results. These were performed using maximum likelihood in PAUP (Heuristic search, 100 random additions and TBR branch swapping) using empirical frequencies and HKY model selected by jModelTest.

RESULTS

Molecular Data

DNA sequences of the four loci were generated for eleven of the twelve specimens included in the phylogenetic analysis (Table 1). The COI fragment consistently failed to amplify for the *C. lugubris* sample using the standard PCR conditions and modified PCR

TABLE 2. Minimum genetic pairwise differences between **C. confusus** and *Colosius* species.

	28S-D2	28S-D3	16S	COI
C. confusus	p-distance			
<i>C. pulcher</i>	0.014	0.014	0.105	0.118
<i>C. propinquus</i>	0.014	0.024	0.111	0.116
<i>C. lugubris</i>	0.010	0.015	0.150	NA
	No. Gap sites (No. INDELS)			
<i>C. pulcher</i>	0 (0)	7 (2)	11 (5)	3 (1)
<i>C. propinquus</i>	0 (0)	4 (2)	12 (6)	3 (1)
<i>C. lugubris</i>	0 (0)	19 (4)	9 (6)	NA

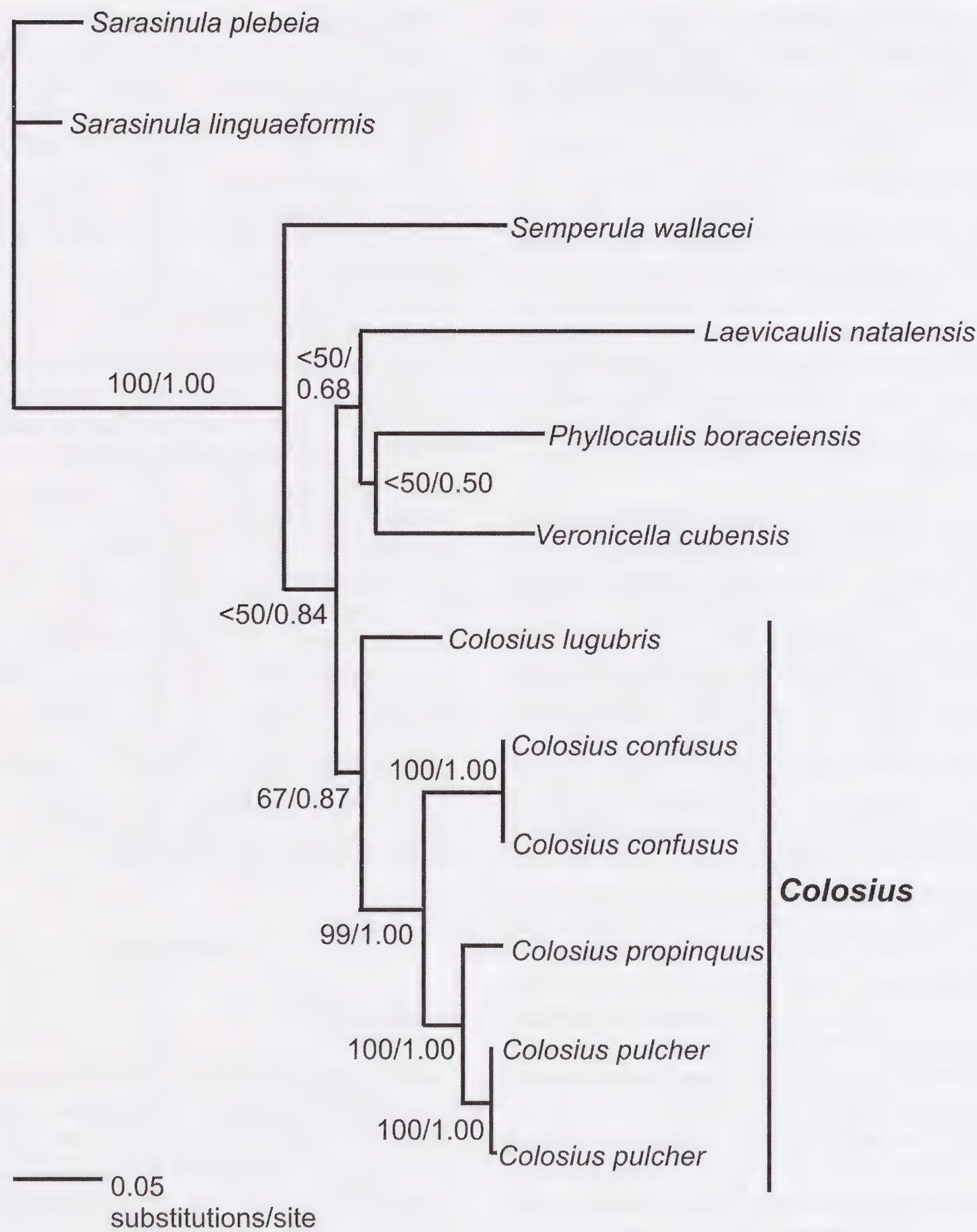


FIG. 1. Best tree identified with branch and bound search for the Maximum Likelihood (score 6023.68061) obtained from the combined cytochrome oxidase I (COI), 16S rRNA, and 28S rRNA) (1593 bp). Numbers above and below nodes are branch support values measured as ML bootstrap and BI posterior probabilities, respectively.

conditions (e.g., increased cycles and lower annealing temperatures). DNA isolated from other *C. lugubris* specimens also failed to amplify the COI and 28S loci. These three *C. lugubris* isolates did generate 16S sequences (JX629303–JX629305) but were not included in further analysis.

As expected for ribosomal genes, segments of the 28S and 16S genes were highly divergent among species and it was difficult to assign

homology to these regions of the alignment. Consequently, those regions were excluded from phylogenetic analysis and model selection but used to calculate pairwise differences among *Colosius* specimens. A final alignment of 1593 bp was generated after concatenation of 28S-D2 (295 bp), 28S-D3 (308 bp), 16S (408 bp), and COI (582 bp). A total of 109 sites were excluded from analyses. These sites are reported here for the concatenated data set and

for each locus in parentheses: 380–423 (28S-D3: 85–129), 618–624 (16S: 15–21), 808–832 (16S: 205–229), 863–867 (16S: 260–264), and 878–905 (16S: 275–302). The percentage of invariant sites in the concatenated data set was highest for the 28S loci (c. 90%) and between 50–60% for the mitochondrial loci.

Uncorrected genetic difference (p-distance) between *Colosius* specimens ranged from 0 to 15.7% for 16S. The minimum distance between different species was 4.1% (between *C. pulcher* and *C. propinquus*). *Colosius lugubris* was the most divergent of the species having minimum p-distances of 14–16% in comparison to the other *Colosius* species. Divergence values for the COI gene ranged from 6.5–11.6% but no data were available from *C. lugubris* for comparison. ***Colosius confusus***, n. sp., had a deletion of an amino acid (3 nucleotides) not shared in the other species. Minimum genetic diversity values between ***C. confusus***, n. sp., and the other *Colosius* species for each gene partition is given in Table 2. This table includes the number of observed gap sites (bases) and insert/deletion (INDEL) events between species.

Only one 16S haplotype was observed in an analysis of 59 ***C. confusus***, n. sp., samples. Analysis of five ***C. confusus*** samples revealed two COI haplotypes that were divergent by 0.2%. Four 16S haplotypes ($\leq 1.2\%$ p-distances) were observed in six samples and two COI haplotypes (0.3% p-distance) were observed in eight samples of *C. pulcher*. Two ***C. confusus***, n. sp., and two *C. pulcher* samples were selected for the phylogenetic analysis (Table 1) in order to consider the diversity found within these species.

A branch and bound search for the Maximum Likelihood tree identified one best tree (score 6023.68061; Fig. 1). This topology was also recovered in the Majority-rule Bayesian Inference tree. Branch support values measured as ML bootstrap and BI posterior probabilities are reported in Figure 1. Each one of the six species of *Colosius* form a monophyletic lineage in the tree. The 28S, 16S, and COI genotypes sequenced from ***C. confusus***, n. sp., samples are distinct from genotypes sequenced from *C. pulcher* and other species of *Colosius* studied (*C. lugubris* and *C. propinquus*). Branch support values are not high for this relationship ($< 80\%$ bootstrap and < 0.90 PP) but it is the best estimate given the available data. According to our phylogenetic results, ***C. confusus***, n. sp., is not the sister-species of *C. pulcher*

(Fig. 1). ***Colosius confusus***, n. sp., is sister to the clade that includes *C. pulcher* and *C. propinquus*. The type species in the genus, *C. lugubris*, is sister to the other *Colosius* species in the study.

ML searches performed on partitioned data resulted in ML trees with different topologies (data not shown). The ML 16S partition tree (score 1837.48372) recovered a *Colosius* topology similar to the combined data set (Fig. 1), but *Semperula wallacei* was included as sister to ***C. confusus***, n. sp. The ML 28S-D2 partition tree (score 610.98971) recovered a monophyletic *Colosius* but with reduced resolution at branches connecting ***C. confusus*** n. sp. to its sister taxon (*C. propinquus* + *C. pulcher*). The 28S-D3 partition tree (score 457.50973) did not recover a monophyletic *Colosius* because *C. lugubris* was not recovered as the sister taxon to the other *Colosius* species. The ML COI partition (score 3212.11170) recovered the topology in Figure 1 but did not include data from *C. lugubris*.

SYSTEMATIC DESCRIPTION

Colosius Thomé, 1975

Colosius confusus, new species
(Figs. 2–7, 12)

Colosius pulcher. Constantino et al., 2010: 1–8 (from Neira, Colombia), *non* Colosi, 1921.

Holotype

ANSP-A22109: Ecuador, Tungurahua, Baños de Agua Santa, W 78°25'24", S 01°23'40", 2,000 m, 11.XII.2004, Col. A. Santillan.

Paratypes

ANSP-A22110: Ecuador, Napo, Cantón Quijos, Bairro Baeza Colonial, N 00°27'32.70", W 077°53'09.78", 1,860 m, 20.IX.2011, Col. S. R. Gomes et al., 1 ex.; CMS-DPE-107'6: Colombia, C'aldas, Neira, 1,700 m, 01.VII.2009, Col. Constantino et al., 7 ex.; QCAZ-M-003744: Ecuador, Chimborazo, Alausí, 26.XII.2000, Col. C. Landeta, 1 ex.; QCAZ-M-003705: Ecuador, Cotopaxi, Otonga, N 00°19'11", W 78°57'00", 2,000 m, 30.VII.2000, Col. D. Alvarado, 1 ex.; QCAZ-M-003813: Ecuador, Napo, Baeza, XI.1985, 1,800 m, Col. Briones, 1 ex.; FMNH- 449321:



Peru, Piura, 11 mi. E Canchaque, on road to Huancabomba, 11.IV.1970, Col. K. Campbell, 1 ex.; MRSN-M492: Ecuador, Pichincha, Volcan Pululahua, N 00°02'16.14", W 078°30'01.56", 2,451 m, 21.IX.2011, Col. D. G. Robinson et al., 1 ex.

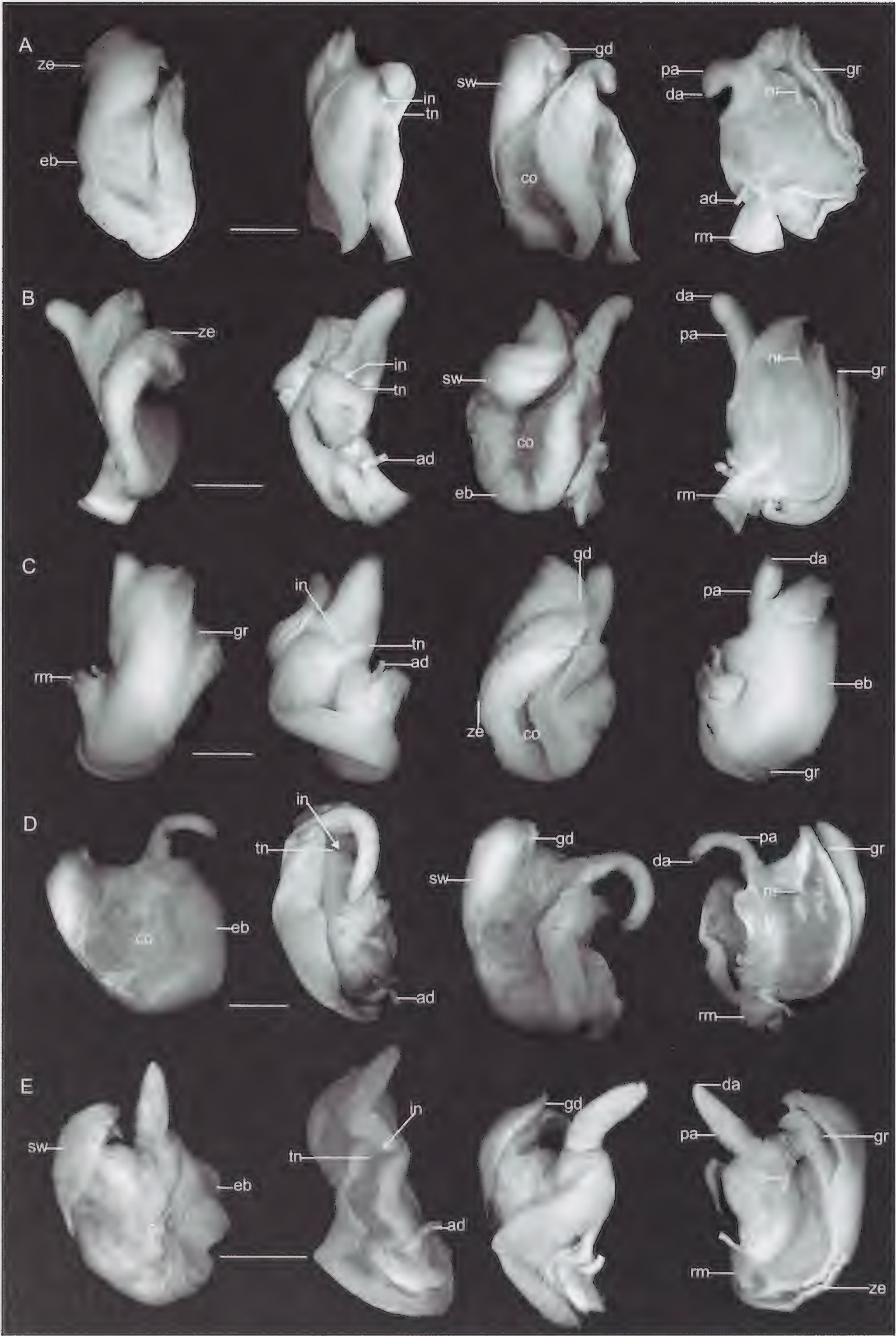
Other Material

QCAZ-M-003657: Ecuador, Imbabura, Caranqui, W 78°07', N 00°19', 3,000 m, 11.XII.1999, Col. C. Lara, 1 ex.; QCAZ-M-003660: Ecuador, Imbabura, Caranqui, N 00°19', W 78°07', 3,000 m, 11.XII.1999, Col. C. Lara, 1 ex.; QCAZ-M-003711: Ecuador, Cotopaxi, Otonga, N 00°25', W 79°00', 2,000 m, 09.I.2003, Col. M. C. Vizcaino, 1 ex.; QCAZ-M-003723: Ecuador, Cotopaxi, Otonga, 2,000 m, 09.I.2003, Col. M. F. Yauri, 1 ex.; QCAZ-M-003731: Ecuador, Cotopaxi, Otonga, N 00°28', W 79°00', 2,000 m, 09.I.2003, Col. M. C. Vizcaino, 1 ex.; QCAZ-M-003745: Ecuador, Chimborazo, Alausí, 02.IX.2000, Col. M. Reinoso, 1 ex.; QCAZ-M-003748: Ecuador, Chimborazo, Alausí, 26.XI.2000, Col. C. Landeta, 1 ex.; QCAZ-M-003750: Ecuador, Chimborazo, Alausí, 23.XI.2000, Col. C. Landeta, 1 ex.; QCAZ-M-003755: Ecuador, Tungurahua, Patate, 23.XI.2000, Col. M. C. Erazo, 1 ex.; QCAZ-M-003758: Ecuador, Bolívar, San Miguel, 20.XII.1991, Col. A. Barragán, 1 ex.; QCAZ-M-003811: Ecuador, Napo, Cuyuja, 18.I.2003, N 00°29'12", W 70°00'48", 2,400 m, Col. R. D. Jadrin, 2 ex.; QCAZ-M-003815: Ecuador, Napo, Cosanga, 07.XII.1986, Col. M. Gavilanes, 2 ex.; QCAZ-M-003820: Ecuador, Napo, Cosanga, XII.1986, Col. J. Naranjo, 1 ex.; QCAZ-M-003821: Ecuador, Napo, El Chaco, 26.I.1993, Col. E. Bejarano, 1 ex.; QCAZ-M-003826: Ecuador, Napo, Cuyuja, 18.I.2003, 2,400 m, N 00°29', W 70°00', Col. F. Gonzales, 1 ex.; QCAZ-M-003828: Ecuador, Napo, Cuyuja, 18.I.2003, 2,400 m, N 00°29', W 70°00', Col. F. Gonzales, 1 ex.; USDA-110422: Hosteria Llacta Termale, Papallacta, Napo,

Ecuador, S 00°21'46.08", W 078°08'5.68", 3,300 m, 20.IX.2011, Col. S. R. Gomes et al., 2 ex.; USDA-110424: Ecuador, Pichincha, Volcán Pululahua, N 00°02'16.14", W 078°30'01.56", 2,451 m, 21.IX.2011, Col. D. G. Robinson et al., 2 ex.; USDA-110425: Ecuador, Embabura, Ibarra, BP Guayabilla, 2,451 m, 01.V.2011, Col. M. Correoso, 2 ex.; USDA 110705: Peru, Piura, Canchaque, S 05°22'19.6", W 079°36'03.2", 1,302 m, 02.VI.2012 Col. S. R. Gomes & V. Jiménez, 9 ex.; USDA 110706: Peru, Piura, Canchaque, S 05°22'16.5", W 079°35'53.9", 1,365 m, 02.VI.2012, Col. S. R. Gomes & V. Jiménez, 1 ex.; USDA-110707: Peru, Piura, Canchaque, S 05°21'22.3", W 079°35'26.8", 1,546 m, 04.VI.2012, Col. S. R. Gomes & V. Jiménez, 6 ex.; USDA-110713: Peru, Amazonas, La Florida, S 05°49'31.0", W 077°58'18.6", 2,220 m, 10.VI.2012, Col. C. Bebbini, 3 ex.; USDA-110715: Peru, Amazonas, La Florida, S 05°49'30.9", W 077°58'2.7", 2,220 m, 09.VI.2012, Col. S. R. Gomes & R. Ramirez, 5 ex.; USDA-110716: Peru, Amazonas, Leymebamba, S 06°42'32.6", W 077°48'17.9", 2,264 m, 10.VI.2012, Col. S. R. Gomes & R. Ramirez, 2 ex.; MUSM-5557: Peru, Amazonas, Leymebamba, S 06°42'18.2", W 077°48'16.4", 10.VI.2012, 2,200 m, Col. S. R. Gomes & R. Ramirez, 1 ex.; USDA-110721: Peru, Amazonas, Leymebamba, S 06°42'57.4", W 077°47'57.3", 2,200 m, 10.VI.2012, Col. S. R. Gomes & R. Ramirez, 2286, 1 ex.; USDA-110722: Peru, Amazonas, Leymebamba, S 06°43'00.2", W 077°48'10.9", 2,316 m, 10.VI.2012, Col. S. R. Gomes & R. Ramirez, 2 ex.; MUSM-5566: Peru, Hacienda Monterezo, Cajamarca, 2,200 m, 17–19.X.2009, Col. L. Figueroa & J. Gragos, 2 ex. Due to the large number of specimens of ***C. confusus***, n. sp., from USDA interceptions (281 to date), mostly from Colombia, the codes related to these specimens are not listed here. They are included at the USDA's Agricultural Quarantine Activity Systems database.

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FIG. 2. External color variation in ***Colosius confusus***, n. sp. A: Dorsal view of an adult specimen from Volcán Pululahua, Pichincha, Ecuador (USDA-110424A); B: Dorsal view of a young specimen intercepted on September 8th, 2010 (USDA-110446); C: Dorsal view of an adult specimen intercepted in Miami from Colombia on March 14th, 2011 (USDA-110448); D: Ventral view of the specimen shown in A; E: Dorsal view of specimen from Cantón Quijos, Napo, Ecuador; F: Dorsal and ventral view of specimens from Canchaque, Piura, Peru, found together at the base of leaves of plantain; G: Dorsal view of specimen from Neira, Manizales, Colombia (Constantino et al., 2010); H: Dorsal view of specimen from Leymebamba, Amazonas, Peru (USDA-110716A); I: Ventral view of the specimens shown in H. ca - bands without or with less spots often present on each side of the lighter line of the notum, that together form sometimes a lozenge-shaped area; bl - lines of black spots that may be present delimiting the lozenge-shaped area; fp - female genital pore; fo - foot; ki - keel; yl - lighter line located longitudinally and centrally on the notum; ys - yellow spots commonly found on the notum.



Etymology

The specific epithet refers to the confusion caused by the misidentification of the species of Veronicellidae with the phallus formed by an expanded muscular base, with two lateral brims, and by a conical and distal papilla. They include ***C. confusus***, *C. pulcher* and new species from Peru, which will be described in a paper currently in preparation. In these species, base and papilla are also delimited from each other by a transversal ridge in the ventral side of the phallus, that also it borders a shallow invagination (Figs. 3, 9, 14).

Diagnosis (Fig. 3)

The species has a phallus formed by an expanded base, with two lateral brims, and by a conical and distal papilla. Both (base and papilla) are delimited from each other by a transversal ridge that also delimits a shallow invagination in the ventral part of the phallus. One of these brims is much more developed than the other and has a deep groove from the phallus base (close to the retractor muscle) to its papilla. On the dorsal part of the phallus, mainly from its $\frac{1}{2}$ distal, a whitish swelling is commonly found along the margin of the groove. On each side of the groove along its entire length, a zigzag raised thread is visible. Numerous fine raised threads are scattered throughout nearly the entire dorsal side of the phallus. A few ridges are, in general, visible near the right distal portion of the groove, close to the papilla on the dorsal side.

External Morphology (Fig. 2)

Notum and hyponota background are brown, ranging from light to dark brown, or reddish-brown. Randomly distinct yellow or beige speckles may occur on this background (Fig. 2A–C, E). In the reddish-brown form, they are almost completely absent or not evident (Fig. 2H). Few black spots may be present, although in general they are sparse. A thin paler line is often centrally and longitudinally present (Fig. 2A, B, H), although its occurrence is variable. It may be absent, non-continuous or visible only near the posterior region of the body, especially in dark brown specimens. A well-defined keel is often present on this line, especially in contracted live specimens (Fig. 2E, G). Two brown bands without or with lighter spots are often present on each side of this paler line, which together can form a lozenge-shaped area (Fig. 2A, B, E, F). This area may be delineated by two lines of black spots (Fig. 2B, H), and barely visible or absent in dark brown specimens or in the reddish-brown form, where only the lines of black spots are present, but not the brown bands (Fig. 2G, H). The hyponota generally have color and intensity similar to the notum, but without the lighter spots observed on the notum (Fig. 2D, F, I). A pale narrow band may be longitudinally present along each side of the foot. The foot is yellow (with different shades of yellow) and is almost the same width as each hyponotum (Fig. 2D, F, I). The female genital pore is located very close to the pedal groove (around 1.5 mm from it) (Fig. 2D). Ocular tentacles are bluish-gray and brown at the tip.

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FIG. 3. Phallus variations of ***Colosius confusus***, n. sp. Five different specimens (A–E) with the phallus in four distinct positions. The first position is the dorsal view, the second is the ventral view and the others are lateral views of the phallus. A: Specimen intercepted from Colombia on August 5th, 2010 (USDA-110447); B: Specimen intercepted from Colombia on June 10th, 2010 (USDA-110449); C: Specimen intercepted from Colombia on March 13th, 2011 (USDA-110448); D: Specimen intercepted from Colombia on November 8th, 2008 (USDA-110450); E: Specimen from Canchaque, Piura, Peru (FMNH-449321). ad- part of the anterior vas deferens penetrating at the phallus base; co - numerous fine raised threads that occupy almost all extension of the dorsal phallus; da- phallus location where the vas deferens opens; eb - expanded phallus base that forms two lateral expansions similar to brims; gd - distal extremity of the groove, often inclined on the phallus in direction of the shallow invagination in the ventral side; gr - longitudinal groove located on the left expanded brim of the phallus; in - shallow invagination delimited by the transversal ridge on the ventral side of the phallus, between the phallus base and its papilla; nr - ridges located near the distal part of the phallus, near the papilla; pa - papilla of the phallus; rm - phallus retractor muscle; sw- whitish swelling throughout the margin of the groove; tn - transversal ridge located between the base of the phallus and its papilla; ze - zig-zag raised threads located on each side of the groove edges. Scale = 2.5 mm.

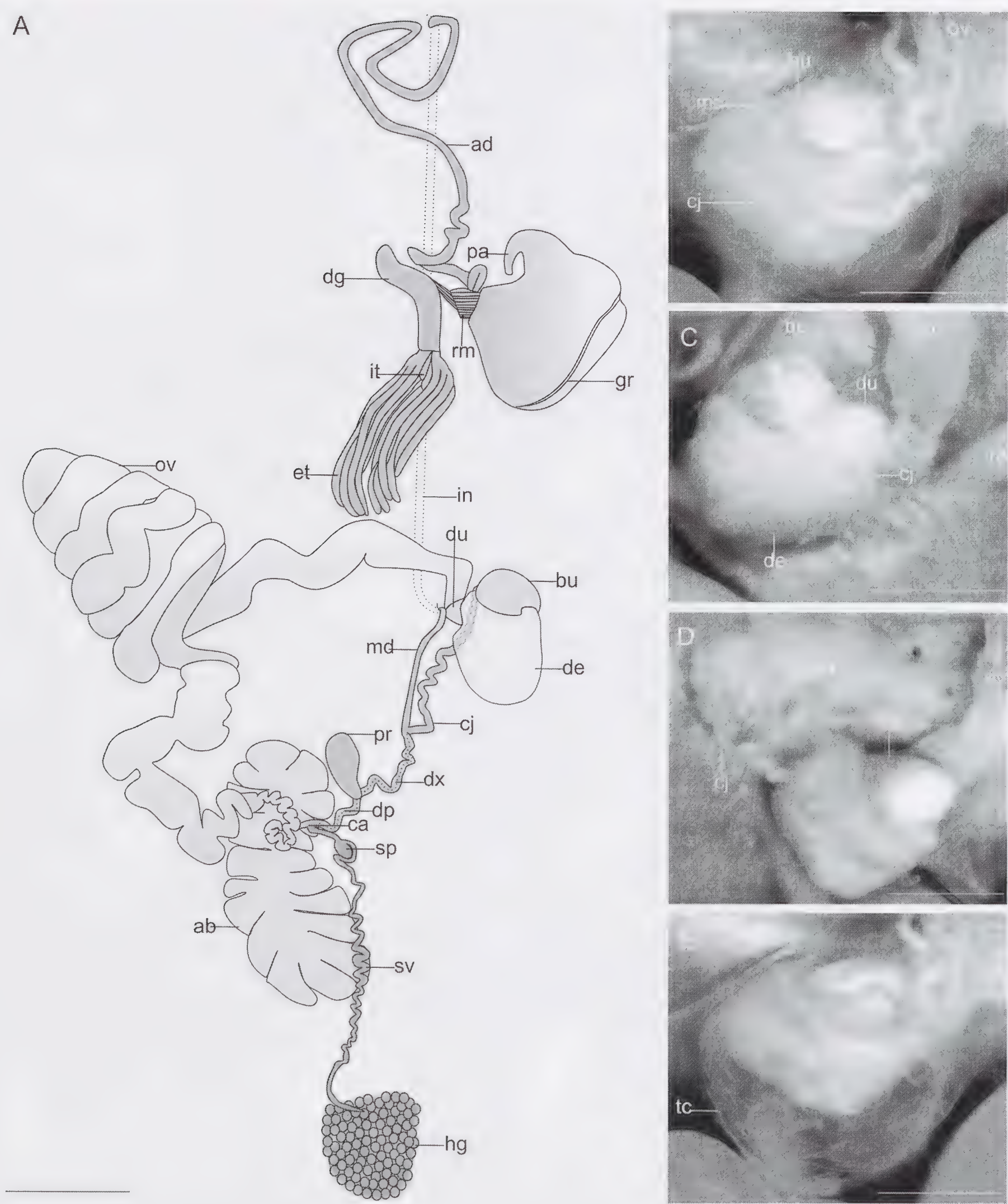


FIG. 4. Reproductive system in *Colosius confusus*, n. sp. A: Drawing of the reproductive system of a specimen intercepted from Colombia on July 15th, 2010 (USDA-1104452); B–E: Detail of the bursa copulatrix in specimen intercepted from Colombia on August 5th, 2010 (USDA-110447). B: Bursa copulatrix showing the canalis junctor strongly connected to the expanded bursa duct; C: Opposite view of B, showing the canalis junctor surrounding the bursa copulatrix duct before penetrating the bursa proper; D: Bursa copulatrix, after the canalis junctor and medium vas deferens disconnected from the duct bursa; E: Bursa copulatrix before the canalis junctor and medium vas deferens, evidencing the tissue that covers the bursa, medium vas deferens and canalis junctor. Ab - albumen gland; ad - anterior vas deferens; ca - carrefour region; bu - bursa copulatrix proper; cj - canalis junctor; de - expanded region of the bursa copulatrix duct; dg - papilla of the digitiform gland; dp - posterior distal vas deferens; du - region not expanded of the bursa copulatrix duct; dx - posterior proximal vas deferens; et - external and long tubules of the digitiform gland; gr - longitudinal phallus groove; hg - hermaphrodite gland; im - course of the vas deferens inside the tegument; it - internal and short tubules of the digitiform gland; md - medium vas deferens; ol - hermaphrodite duct; ov - oviduct; pa - papilla of the phallus; pr - prostate; rm - retractor muscle; sp - fertilization complex; sv - seminal vesicle; tc - tissue that cover the bursa copulatrix and duct around it. Scale = 2.5 mm.

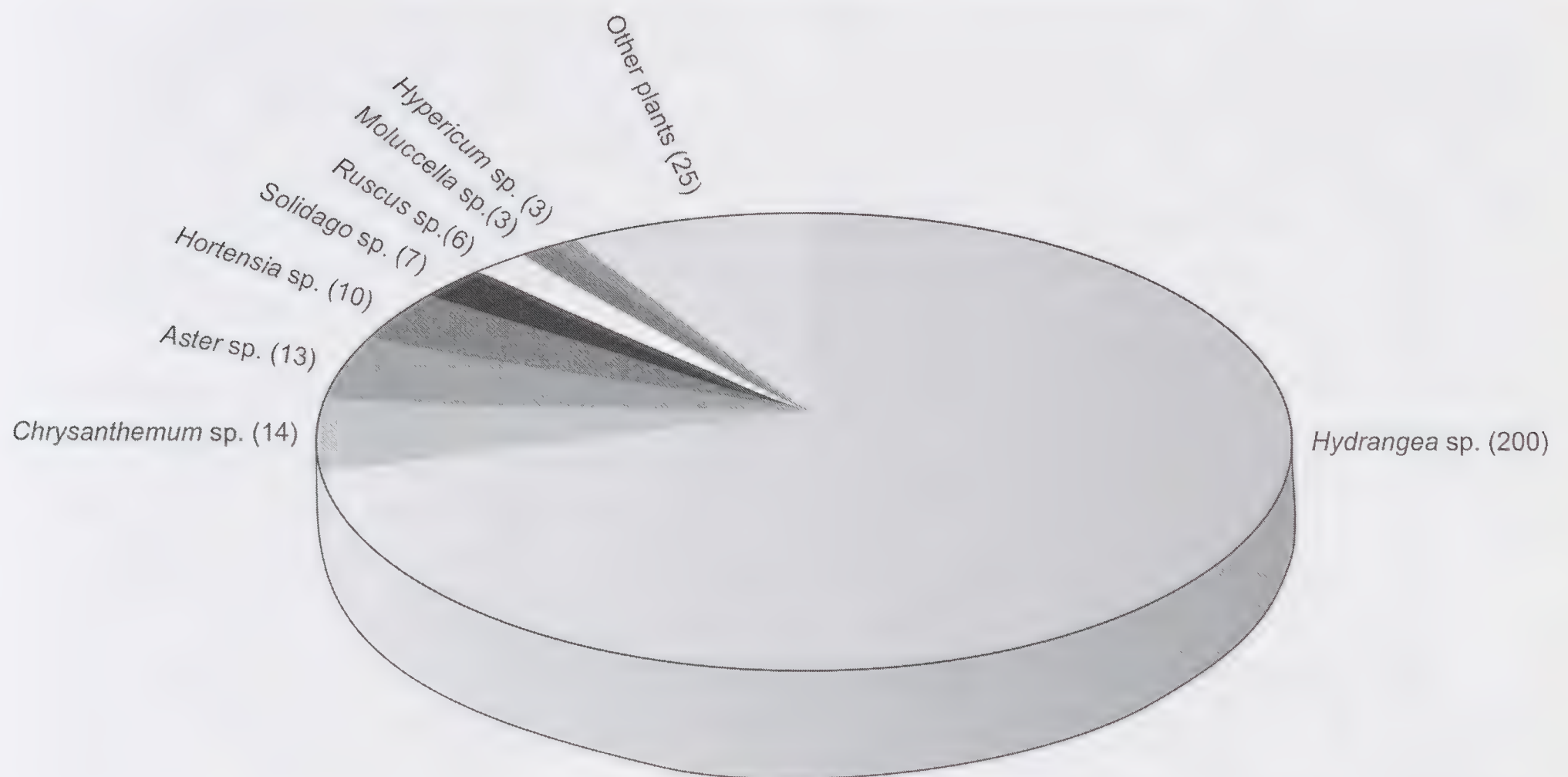


FIG. 5. Imports from the United States from 2001 to 2011, associated with interceptions of *Colosius confusus*, n. sp.

Internal Morphology

Pedal gland, salivary glands, pedal and pallial nerves and posterior pedal artery in *C. confusus*, n. sp., are morphologically similar to most species of Veronicellidae from Central and South America (Thomé, 1975). Diagnostic features are found in the reproductive system, in particular, bursa copulatrix and phallus.

Reproductive System (Figs. 3, 4, 13): All veronicellids are hermaphroditic and have an anteriorly located male genital pore and medially located female pore. A good description and illustration of the reproductive system in the family Veronicellidae is found in Thomé et al. (2006).

The phallus in *C. confusus*, n. sp., is very complex and variable (Fig. 3), assuming different conformations according to its distention, development level and the different folding. It is enveloped by a diaphanous muscular sheath (connected to the sheath of the papilla of the digitiform gland of same thickness, with which it shares a common atrium), and is strongly connected to the tegument by a short and thick retractor muscle. It is leaf-like with an expanded base, with two lateral brims and by a distally located papilla, where the vas deferens opens at its extremity. The right brim is more developed and has a deep longitudinal groove from its base near

the retractor muscle to close the papilla. A zigzag-shaped raised thread is present along the entire length of each side of the groove. The groove is often more expanded near the phallus base and distally near the papilla. On the dorsal part of the phallus, mainly from its $\frac{1}{2}$ distal, it is common a whitish swelling that is throughout the margin of the groove. A transversal ridge is present on the ventral side of the phallus, delimiting the base from the phallus papilla. This ridge also delimits a shallow invagination on the same side, which is variable in depth. The distal portion of the groove is often inclined on the phallus in the direction of the invagination. Numerous fine raised threads occupy nearly the entire extension of the opposite side of the phallus (dorsal side). A few ridges may also be evident next to the distal portion of the groove, near the papilla on the dorsal side. The papilla is conical and can vary in length, width, and proportion relative to the base.

Accessory to the phallus is the digitiform gland (analogous to the sarcobellum of some stylommatophorans, as described in Reise et al. (2011), which has tubules differentiated in two kinds: short internal tubules, and long external tubules that are very branched at the base and enclosed the shorter ones (Figs. 4A, 13). The internal tubules are generally lighter and thinner. In a sample of 40 specimens 1–3 internal tubules and 15–23 long



FIG. 6. Distribution of *Colosius confusus*, n. sp., Lighter red regions of Ecuador and Colombia correspond to regions of cut-flower production.

external tubules were found. The papilla of the digitiform gland is conical, narrow, long and tapered in the extremity.

In *C. confusus*, n. sp., after the medium deferens passes the prostate, which is pear-shaped and poorly developed (in general smaller than the bursa copulatrix), it continues free for a relatively long distance (Fig. 4A), before it penetrates in the tegument, near the duct base of the bursa copulatrix. The canalis junctor is long and sinuous and strongly compacted to the expanded bursa duct (Fig. 4B, D). In most specimens, the duct can seem to run inside the bursa duct. In younger specimens it is more easily separated from the bursa duct and more recognizable. The canalis junctor crosses attached to the bursa duct from its distal to the proximal region (near the female pore),

where it surrounds the right side of the duct, before it penetrates in the bursa copulatrix proper (Fig. 4C).

The bursa copulatrix is initially formed by a short non-expanded portion, close to the female genital pore. After, the duct is modified in a large lateral expansion that can be confused with the bursa proper, which is much smaller and has much thinner walls. The bursa duct internally has very developed pilasters that probably are responsible for expanding this region during mating, receiving and accommodating the developed lateral expansions of the phallus. Beyond this expansion there is a narrowing, which has width and length similar to that of the papilla of the phallus, which probably receives it. This portion is connected to the bursa proper, formed by a round sac with thin walls, where the sperm is

received and the excess probably destroyed (= gameolitic gland). The bursa appears to be laterally connected to the duct bursa, although it can be completely separate from the duct, after removing the tissue covering the bursa region (Fig. 4B, E). An accessory gland near the female genital pore is absent in *C. confusus*, n. sp.

Pedal Gland: The pedal gland, which is located on the anterior extremity of the sole under the head, is short and straight. Its posterior extremity is a little more expanded and is strongly connected to the pedal gland artery, at the height of the periesophagic nervous ring. In general, it is possible to distinguish two different areas in the pedal gland: an external lighter and an internal yellowish. The pedal gland opening is v-shaped.

Salivary Gland: Salivary gland is formed by large, individualized acini.

Pedal and Pallial Nerves: One pair of pallial and one pair of pedal nerves run parallel from the central nervous system towards the posterior region of the body. They are paired for a short distance, until around the pericardium height. From there, they diverge in an acute angle and, then run parallel and away from each other to the posterior region of the body.

Posterior Pedal Artery: The posterior pedal artery originates at the height of the central nervous system, running free between the pairs of pedal and pallial nerves for a short distance before penetrating the tegument, around the pericardium height.

Habitat

Specimens recently collected in Ecuador (USDA-110422, USDA-110424) were found in disturbed areas close to crops in Volcán Pululahua (Pichincha) and in an urban area, close to houses in Cantón Quijos (Napo), under rocks and wood. Specimens from Colombia and Ecuador intercepted were found in shipments of *Hydrangea* sp. (Fig. 5), but also with various other species such as *Chrysanthemum* sp., *Aster* sp., *Hortensia* sp., *Solidago* sp., *Ruscus* sp., *Moluccella* sp. and *Hypericum* sp. A few interceptions (two or less) also occurred in *Acacia* sp., *Alstroemeria* sp., *Eucalyptus* sp., *Gerbera* sp., *Gypsophila* sp., *Jatropha* sp., *Lisianthus* sp., *Mentha* sp., *Ornithogalum* sp., *Pittosporum* sp., *Polianthes* sp., *Rosmarinus*

spp. and *Veronica* sp. The species is recorded as *C. pulcher* causing damage to coffee crops in Colombia (Constantino et al., 2010). In Peru, the species was found attacking coffee in Canchaque, Piura. The animals use the base of plantain leaves to be hidden during the day, which is cultivated with coffee. In Amazonas, a different reddish-brown form was found living in urban areas, where other native species of slugs were also found, although not causing agriculture damage. Records of occurrence of this species, in the three countries, range from 1,500 m to 3,123 m of altitude.

Occurrence (Fig. 6)

Colombia: Caldas Department: Neira.

Ecuador: Imbabura Province: Caranqui, Ibarra; Pichincha Province: Volcán Pululahua; Napo Province: Cuyuja, Baeza, Cosanga, El Chaco, Cantón Quijos; Cotopaxi Province: Otonga; Tungurahua Province: Patate; Bolivar Province: San Miguel; Chimborazo Province: Alausí.

Peru: Piura Department: Canchaque; Amazonas Department: La Florida, Leymebamba; Cajamarca Department: Fazenda Monterezo.

Two specimens were intercepted from Ecuador. The others were intercepted from Colombia. Most of Colombia's cut flower production is located around Bogotá, with small production areas in the regions around Rionegro and Antioquia Medellin and Cali (USITC, 2003). In Ecuador, flower crops are concentrated in Pichincha (71%), Cotopaxi (22.2%), Imbabura (3.9%), Azuay (1.2%) and others (1.4%) (Acción Ecológica, 2000).

Genetic Variability

The genetic diversity within *C. confusus*, n. sp., is relatively low based on estimates using segments of 16S and COI mitochondrial DNA. This indicates that these regions could be useful for species diagnosis and delimiting species boundaries. The 28S, 16S, and COI genotypes sequenced from *C. confusus*, n. sp., samples are distinct from genotypes sequenced from *C. pulcher* and other species of *Colosius* studied, *C. lugubris* and *C. propinquus*. Based on phylogenetic analyses, *C. confusus*, n. sp., is a distinct lineage within the genus *Colosius* and not the sister taxon to *C. pulcher*. In comparison with *C. pulcher*, the *C. confusus*, n. sp., specimens can be identified based on unique nucleotide insertion and deletion events for 28S, 16S, and COI genes.

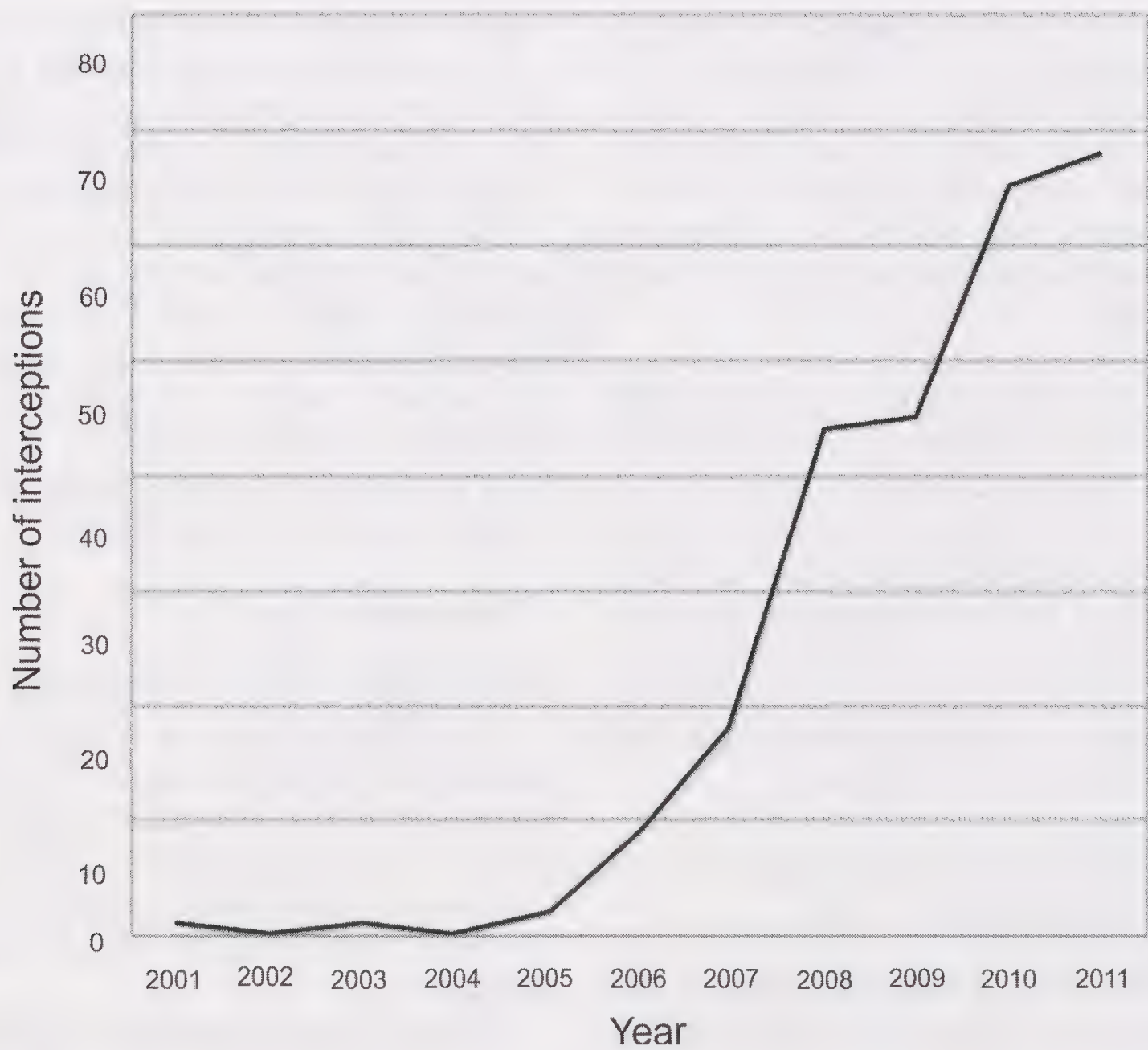


FIG. 7. Interceptions of *Colosius confusus*, n. sp., by year (2001–2011), by federal agricultural inspectors.

Remarks

The first record of *C. confusus*, n. sp., intercepted by federal agricultural inspectors was on April 6th, 2001 (USDA-110469), in Miami on *Gypsophila* sp. from Ecuador. In 2003, the species is intercepted for the first time from Colombia on *Moluccella* sp. In 2004 interceptions increased rapidly in imports from Colombia (Fig. 7), especially on *Hydrangea* sp. (Fig. 5).

Colosius pulcher (Colosi, 1921)
(Figs. 11–14)

Vaginula pulchra Colosi, 1921: 157.

Vaginula pulchra Colosi: Colosi, 1922: 496–498.

Vaginula pulchra Colosi: Thomé, 1970a: 23–25 (lectotype, paralectotype and type-locality designated).

Non Angustipes (Angustipes) pulcher of Colosi: Kraus, 1953: 63 (from Hacienda Taulis, Cajamarca Department, Peru).

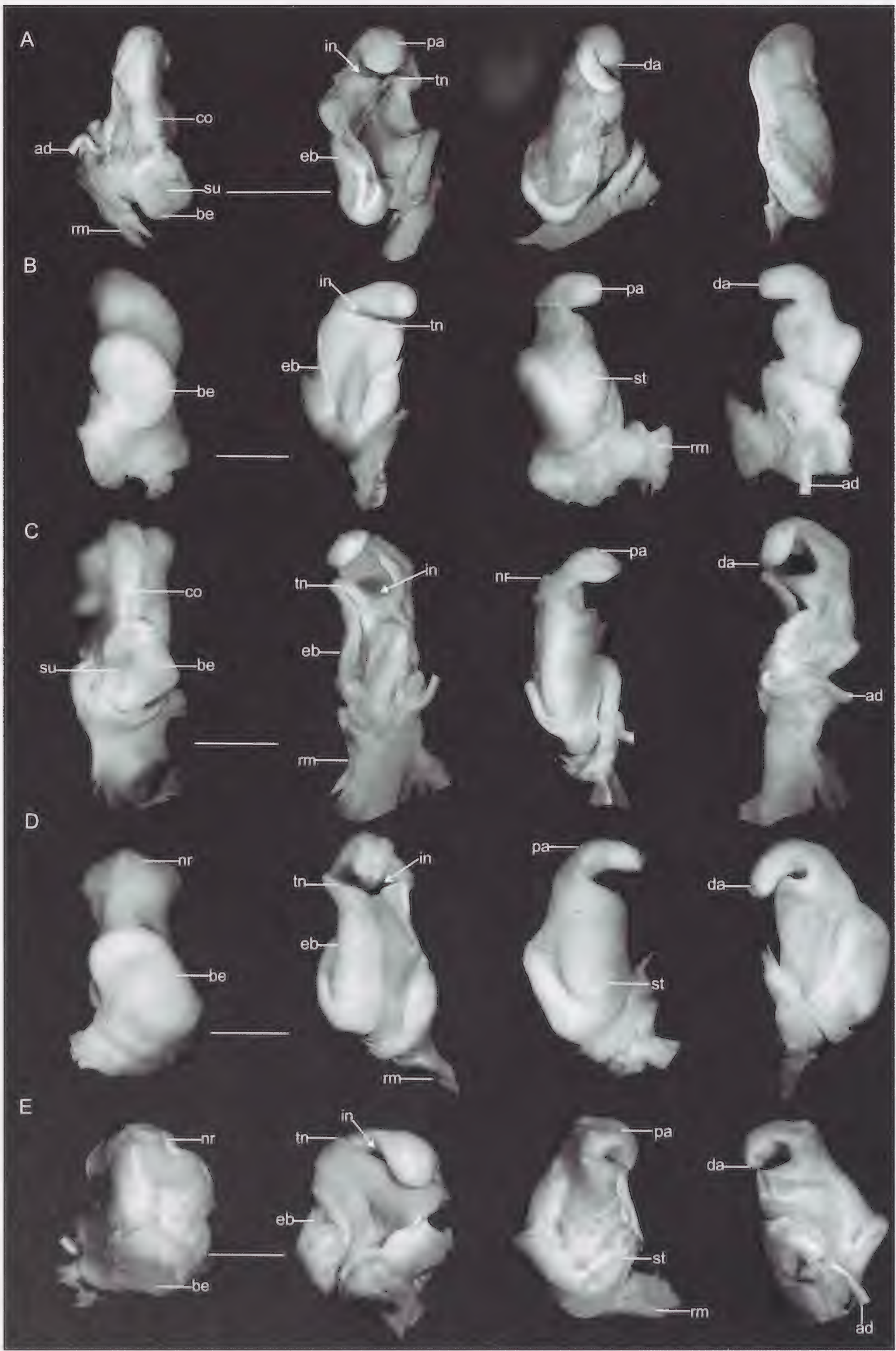
Colosius pulchrus: Thomé, 1975: 13; 1993: 72.

Non Colosius pulcher of Colosi: Thomé et al. 2001: 70–71 (from different localities from Peru).

Non Colosius pulcher of Colosi: Thomé et al. 1997: 522; ANSPA1092 (from “St. Domingo” – Dominican Republic).

FIG. 8. External coloration variation in adult specimens of *Colosius pulcher*. A: Dorsal view of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110419B); B: Dorsal view of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110420B); C: Dorsal view of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110420C); D: Ventral view of the specimen shown in A (USDA-110419B); E: Ventral view of the specimen shown in B (USDA-110420B); F: Ventral view of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110420A); G: Ventral view of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110421B); H: Dorsal view of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110419C); I: Dorsal view of the specimen shown in F (USDA-110420A). bs-black spots distributed on the entire notum; fp-female genital pore; fo-foot; yl- lighter line located longitudinally and centrally on the notum; ys-yellow spots that are distributed irregularly on the notum.





Colosius pulcher: Thomé et al., 1997: 522; USNM 769142.

Non Colosius pulcher of Colosi: Constantino et al., 2010: 1–8 (from Neira, Colombia).

Material Examined

QCZA-M-003647: Ecuador, Imbabura, San Pablo, N 00°15', W 078°10', 29.XII.1999. Col. G. Maldonado, 1 ex.; QCZA-M-003654: Ecuador, Imbabura, San Pablo, 19–20.I.2002., Col. F. Maza, 1 ex.; QCZA-M-003655: Ecuador, Imbabura, Lago San Pablo, 27.XII.1988, Col. U. Benítez, 1 ex.; QCZA-M-003662: Ecuador, Imbabura, Lita, 01.X.2001, Col. P. Villamar, 1 ex.; QCZA-M-003669: Ecuador, Imbabura, Peguche, 07.I.2001, Col. G. Buitrón, 1 ex.; QCZA-M-003672: Ecuador, Pichincha, Santo Domingo, 16.I.1993, Col. L. Torres, 1 ex.; QCZA-M-003676: Ecuador, Pichincha, Aloag, 1.XI.1983, Col. C. Padrez, 1 ex.; QCZA-M-003679: Ecuador, Pichincha, Santo Domingo, 4.XI.1983, Col. M. V. Gangotena, 2 ex.; QCZA-M-003697: Ecuador, Napo, Papallacta, 3,200 m, 8.I.1995, Col. K. Suárez, 1 ex.; QCZA-M-003698: Ecuador, Sucumbíos, Oyacachi, 3,200 m, 2.XI.1992, Col. L. Della Torre, 1 ex.; QCZA-M-003699: Ecuador, Napo, Papallacta, 3,200 m, 8.I.1995, Col. K. Suárez, 1 ex.; QCZA-M-003703: Ecuador, Cotopaxi, Iliniza Sur, 3,400 m, 22.I.1989, Col. V. Nuñez, 10 ex.; QCZA-M-003706: Ecuador, Cotopaxi, Pujilí, XII.1993, Col. J. Arroyo, 1 ex.; QCZA-M-003707: Ecuador, Cotopaxi, Iliniza Sur, 21.I.1989, Col. F. Haro, 1 ex.; QCZA-M-003708: Ecuador, Cotopaxi, Boliche, 31.XII.1987, Col. M. Peñaherrera, 3 ex.; QCZA-M-003712: Ecuador, Cotopaxi, Iliniza Sur, 21.I.1989, Col. F. Haro, 1 ex.; QCZA-M-003713: Ecuador, Cotopaxi, Pastocalle, 3,900 m, 12.XII.1987, Col. M. Larrea, 1 ex.; QCZA-M-003714: Ecuador, Cotopaxi, Parque Nacional, 3,280 m, 04.XI.1995, Col. L. Torres,

1 ex.; QCZA-M-003718: Ecuador, Cotopaxi, Iliniza Sur, 3,400 m, 12.I.1989, Col. C. Ayala, 1 ex.; QCZA-M-003719: Ecuador, Cotopaxi, Pastocalle, 3,406 m, 12.XI.1987, Col. M. Larrea, 3 ex.; QCZA-M-003720: Ecuador, Cotopaxi, Pastocalle, 3,400 m, 12.XII.1987, Col. M. Larrea, 2 ex.; QCZA-M-003721: Ecuador, Cotopaxi, Latacumba, IX.1981, Col. Gonore, 1 ex.; QCZA-M-003724: Ecuador, Cotopaxi, Latacumba, S 00°55'52", W 79°36'46", 2,770 m, 26.II.2000, Col. D. Alvarado, 1 ex.; QCZA-M-003728: Ecuador, Cotopaxi, Iliniza Sur, 3,400 m, 21.I.1989, Col. C. Ayala, 1 ex.; QCZA-M-003729: Ecuador, Cotopaxi, Parque Nacional, 3,280 m, 04.IX.1995, Col. A. Muñoz, 1 ex.; QCZA-M-003733: Ecuador, Cotopaxi, Iliniza Sur, 3,400 m, 21.I.1989, Col. C. Ayala, 1 ex.; QCZA-M-003735: Ecuador, Cotopaxi, Iliniza Sur, 3,400 m, 21.I.1989, Col. C. Ayala, 1 ex.; QCZA-M-003738: Ecuador, Cotopaxi, Iliniza Sur, 3,400 m, 21.I.1989, Col. C. Ayala, 1 ex.; QCZA-M-003743: Ecuador, Chimborazo, Penipe-Puela, 2,300 m, 23.I.1986, Col. X.J. Jaurieth, 2 ex.; QCZA-M-003754: Ecuador, Carchi, San Gabriel, 27.XI.1989, Col. C. Puertas, 1 ex.; QCZA-M-003756: Ecuador, Carchi, San Gabriel, 27.IX.1989, Col. P. Enriquez, 1 ex.; QCZA-M-003762: Ecuador, Pichincha, Quito, 2,850 m, 02.I.1986, Col. C. Fierro, 1 ex.; QCZA-M-003763: Ecuador, Pichincha, Quito, 2,800 m, Col. Briones, 1 ex.; QCZA-M-003772: Ecuador, Pichincha, Quito, S 00°11'22", W 78°29'38", 20.XI.2000, Col. X. Haro, 1 ex.; QCZA-M-003774: Ecuador, Napo, Papallacta, S 00°22', W 78°09', 3,200 m, 17.I.1999, Col. S. Castelo, 1 ex.; QCZA-M-003776: Ecuador, Pichincha, Quito, S 00°11'22", W 78°29'38", 2,750 m, 05.I.2003, Col. D. Cevallos, 1 ex.; QCZA-M-003779: Ecuador, Pichincha, Pululahua, 15.IX.1989, Col. V. Perez, 1 ex.; QCZA-M-003782: Ecuador, Pichincha, Quito, 2,800 m, 04.I.1993, Col. G. Fletcher, 1 ex.; QCZA-M-003789: Ecuador, Pichincha, Quito,

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FIG. 9. Phallus variation in *Colosius pulcher*. Five different specimens (A–E) with the phallus in four distinct positions. The first position is the dorsal view, the second is the ventral view and the others are lateral views of the phallus. A: Specimen from Quito, collected by Dr. Festa (between 1895–1898), part of the material used by Colosi (1921) to propose *C. pulcher* (MRSN-3232); B: Specimen intercepted by USDA from Ecuador in 1969 in Miami (USNM-769142); C: Specimen from Mount Cotopaxi, Ecuador (FMNH-255555A); D: Specimen from Paschoa, Pichincha, Ecuador (USDA-110420D); E: Specimen from Quito (ZMUC, specimen A). ad - part of the anterior vas deferens penetrating the phallus base; be - oval to rectangular swelling found on the basal region of the phallus; co - longitudinal and central swelling often visible on the phallus; da - location on the phallus where the vas deferens opens; eb - phallus expanded base that forms two expansions similar to lateral brims; in - shallow invagination delimited by a transverse ridge on the ventral side of the phallus, between its base and papilla; pa - phallus papilla; rm - phallus retractor muscle; su - thin grooves can be seen on the basal swelling, similar to cracking; st - ridges that may be present on the left side of the expanded phallus base; tn - transversal ridge located between the base of the phallus and its papilla. Scale = 2.5 mm.

I.1983, Col. G. Paz, 1 ex.; QCZA-M-003790: Ecuador, Pichincha, Quito, 22.I.1990, Col. E. Guerrón, 1 ex.; QCZA-M-003791: Ecuador, Pichincha, Quito, 2,800 m, I.1986, Col. Brione, 1 ex.; QCZA-M-003792: Ecuador, Pichincha, Quito, S 0°9', W 78°26', 02.XI.2002, Col. A.V. Carvajal, 1 ex.; QCZA-M-003793: Ecuador, Pichincha, Quito, 2,800 m, 14.XI.1984, Col. L. Duque, 1 ex.; QCZA-M-003794: Ecuador, Pichincha, Quito, XII.1986, Col. G. Medina, 1 ex.; QCZA-M-003795: Ecuador, Pichincha, Calderon, 24.XI.1989, Col. L. Schel, 1 ex.; QCZA-M-003799: Ecuador, Pichincha, Pifo-Baeza, 1,900 m, 30.XI.1985, Col. A. Izurieta, 1 ex.; QCZA-M-003801: Ecuador, Napo, Papallacta, 13.XII.1987, Col. A. Cordova, 5 ex.; QCZA-M-003802: Ecuador, Napo, Papallacta, 12.XII.1987, Col. A. Cordova, 3 ex.; QCZA-M-003803: Ecuador, Napo, Papallacta, 3,200 m, S 00°22', W 78°22', 17.I.1999, Col. S. Castelo, 1 ex.; QCZA-M-003804: Ecuador, Napo, Papallacta, 18.XII.1987, Col. A. Cordova, 1 ex.; QCZA-M-003805: Ecuador, Napo, Papallacta, 13.XII.1987, Col. A. Córdoba, 4 ex.; QCZA-M-003806: Ecuador, Napo, Archidona, I.1987, Col. G. Medina, 1 ex.; QCZA-M-003807: Ecuador, Napo, Archidona, I.1987, Col. G. Medina, 1 ex.; QCZA-M-003808: Ecuador, Napo, Borja, 12.X.1990, Col. G. Aguilar, 1 ex.; QCZA-M-003809: Ecuador, Napo, Papallacta, 07.I.1989, Col. P. Coial, 1 ex.; QCZA-M-003810: Ecuador, Napo, Papallacta, 04.XII.1987, Col. A. Córdoba, 5 ex.; QCZA-M-003812: Ecuador, Napo, Papallacta, 13.XII.1987, Col. A. Córdoba, 1 ex.; QCZA-M-003814: Ecuador, Napo, Papallacta, 3,200 m, 08.I.1995, Col. K. Suárez, 1 ex.; QCZA-M-003818: Ecuador, Napo, Papallacta, 09.X.1987, Col. P. O. Jedo, 1 ex.; QCZA-M-003819: Ecuador, Napo, Papallacta, 25.I.1989, Col. C. Molina, 1 ex.; QCZA-M-003823: Ecuador, Napo, Papallacta, 07.I.1989, Col. P. Coral, 1 ex.; QCZA-M-003825: Ecuador, Napo, Papallacta, 3,159 m, 21.X.1995, Col. R. Paladines, 1 ex.; USDA-110419: Ecuador, Pichincha, Pasocha, S 00°25'12.38", W 078°31'11.64", 2,812 m, 19.IX.2011, Col. David et al., 3 ex.; USDA-110420: Ecuador, Pichincha, Pasocha, S 00°23'08.70", W 078°24'58.26", 2,736 m, 19.IX.2011, Col. D. G. Robinson et al., 30 ex.; USDA-110421: Hosteria Llacta Termale, Papallacta, Napo, Ecuador, S 00°21'46.08", W 078°08'55.68", 3,300 m, 20.IX.2011, Col. S. R. Gomes et al., 3 ex.; USDA-110423: Volcán Pululahua, Pichincha, Ecuador, N 00°02'16.43", W 078°30'01.56", 2,451 m, 21.IX.2011, Col. D. G. Robinson et al., 7 ex.; FMNH-255555: 1 mi. NE Mt. Cotopaxi, 2,956 m, 03.V.1970, Col. K. Campbell, 8 ex.; USNM-769142: Ecuador, USDA

interception on bromeliads, 18.Mar.1969, 1 ex.; MRSN-3232: Ecuador, Quito, 2,850 m, Col. Festa (paralectotypes), 3 ex.; ZMUC: Ecuador, Quito, Col. Banget, 8 ex.

Diagnosis (Fig. 9)

Species with a phallus formed by an expanded and muscular base that forms two lateral brims, and by a distal and conical papilla. Both (base and papilla) are delimited from each other by a transversal ridge on the ventral portion of the phallus, which also delimit a shallow invagination. An oval to rectangular swelling is present on the base on the dorsal side of the phallus. Ridges are also often on the right lateral expansion of the phallus.

External Morphology (Fig. 8)

Adult specimens are of a medium size, with an extended length up to 8–9 cm. The notum and hyponota can have different shades of brown (Fig. 8A, B, G, H), ranging from dark (Fig. 8A) to light brown (Fig. 8B), but also yellowish-brown (Fig. 8C) and reddish-brown (Fig. 8I). Beige or yellow and black spots are found on this background. In general they are scattered over the entire notum without forming any specific pattern. Such spots may vary in quantity, defining a more homogenous coloration for the notum (Fig. 8A, I). A pale, thin line, located medially and longitudinally along the notum is almost always present, but it may also be absent or not continuous (Fig. 8A–C, I, M). Rarely two black bands are present on either side of the pale line. The coloration of the hyponota generally is the same as the notum background (Fig. 8D–F), but it can also be lighter. The hyponota often become paler immediately adjacent to the foot. The foot is light yellow and has approximately the same width as each hyponotum. The genital pore is located very close to the pedal groove (Fig. 8F). Ocular tentacles are bluish-gray or beige.

Internal Morphology

Pedal gland, salivary glands, pedal and pallial nerves and pedal artery have the same patterns described for *C. confusus*, n. sp.

A thin and translucent muscular sheath envelops the phallus of *C. pulcher*. The sheath is somewhat thicker than seen in *C. confusus*, n. sp. It is strongly connected to the tegument by a short, thick retractor muscle. It is leaf-like, having an expanded base that forms two

A

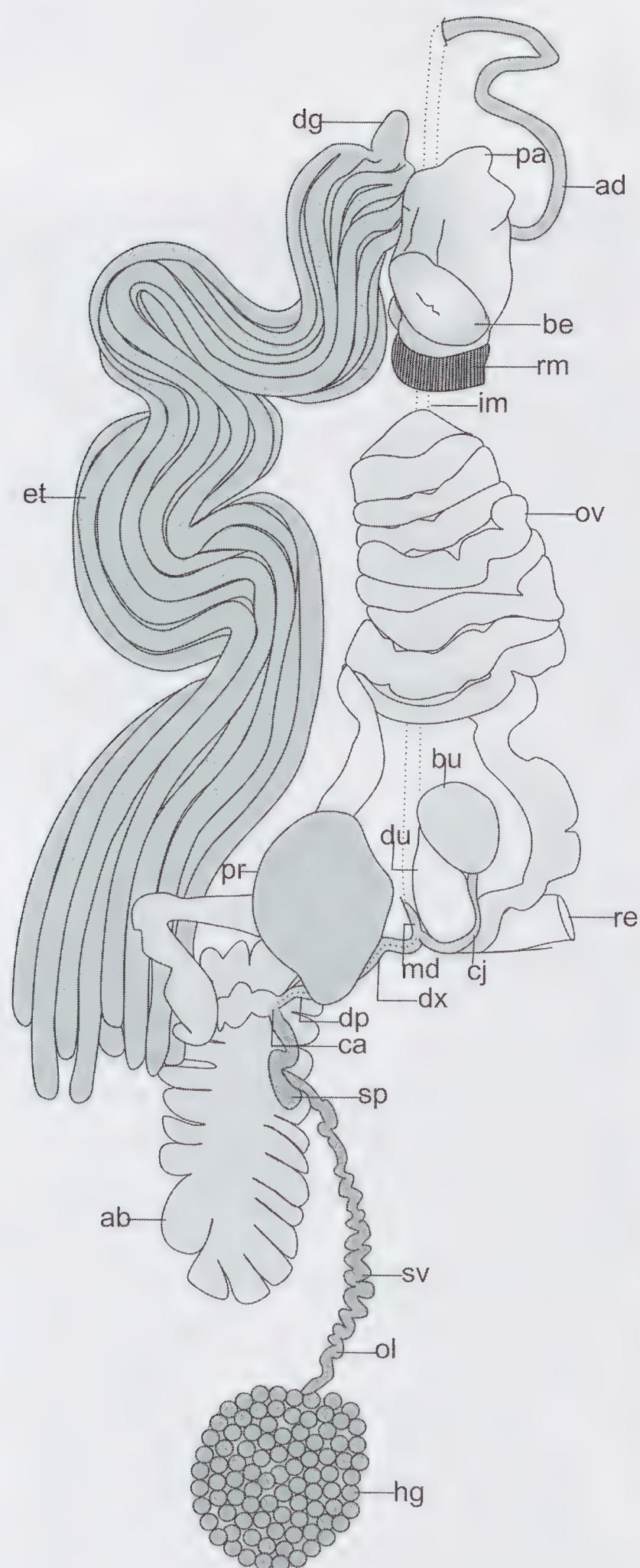


FIG. 10. Reproductive system in *Colosius pulcher*. A: Drawing of the complete reproductive system of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110420C); B–E: Detail of the bursa copulatrix in specimen from Papallacta, Napo, Ecuador (USDA-110421C). B: Bursa copulatrix prior to having the tissue around it, the canalis junctor and the medium vas deferens removed; C: Opposite view of bursa copulatrix shown in B, showing the canalis junctor surrounding the right side of the base of the bursa copulatrix duct before it penetrates the bursa proper, giving the wrong impression that it is penetrating the duct of the bursa; D: Bursa copulatrix and canalis junctor, after surrounding tissue has been removed, showing the penetration of the canalis junctor on the bursa proper; E: Bursa copulatrix region showing the spongy aspect of the tissue (shown compacted in picture B) located near the bursa copulatrix base. Ab - albumen gland; ad - anterior vas deferens; be - swelling found on the phallus base; ca - carrefour; cj - canalis junctor; bu - bursa copulatrix proper; du - bursa copulatrix duct; dg - papilla of the digitiform gland; dp - posterior distal vas deferens; dx - posterior proximal vas deferens; et - external and long tubules of the digitiform gland; im - vas deferens course inside the tegument; md - medial vas deferens; ol - hermaphrodite duct; ov - oviduct; pa - phallus papilla; pr - prostate gland; re - part of the rectum; rm - phallus retractor muscle; sp - fertilization complex; sv - seminal vesicle; hg - hermaphrodite gland (= gonad). Scale = 2.5 mm.



FIG. 11. Distribution of *Colosius pulcher*.

lateral brims on each side of the phallus, and a conical papilla, where at its distal extremity the vas deferens opens. The lateral expansions are less developed than those of *C. confusus*, n. sp., and are folded in the direction of the concave ventral side. On the ventral side there is a transversal ridge between the phallus base and its papilla, where there is a shallow invagination, which varies in depth between specimens. The papilla of the phallus is conic and short, less variable in length, width and proportion than seen in *C. confusus*, n. sp. It is often curved in the direction of the shallow invagination. On the dorsal side of the right expanded brim (opposite side of that where the vas deferens opens) ridges are commonly found, which range in degree of development. In addition, ridges may also be present at the boundary between the expanded base of the phallus and its papilla. In the basal region of the phallus there is an oval to rectangular swelling. Some thin grooves may be seen on this swelling, similar to cracking. In general, the phallus in *C. pulcher* is less variable than in *C. confusus*, n. sp. Variations are related to its width and degree of development and folding of their lateral brims. The shape, size

and exact position of the swelling found at the base of the phallus and degree of development of the ridges on the dorsum of the phallus are also variable.

The digitiform gland has internal short and external long and basally branched tubules as in *C. confusus*, n. sp. The papilla of the digitiform gland is conical and short. The digitiform gland is often longer than in *C. pulcher*. In a sample of 40 specimens, the external long tubules ranged from 17 to 24 and the short internal tubules from 5 to 12. The papilla is conical, short and triangular (Figs. 4, 10, 13).

The bursa copulatrix duct in *C. pulcher* (Fig. 10) has a medium length, is thick and has internally numerous pilasters, but it is not laterally expanded as in *C. confusus*, n. sp. The canalis junctor is long, but less sinuous and thicker than seen in *C. confusus*, n. sp. It is also strongly connected to the bursa copulatrix duct. After it runs connected on the entire length of the bursa duct, it surrounds the right base of the bursa duct before entering the base of the bursa copulatrix. The bursa proper is round to oval and composed of non-muscular, non-glandular tissue. Between the bursa duct and the rectum is a glandular structure that is

constituted internally by spongy compressed tissue (Fig. 10C, E). In *C. pulcher*, the prostate is more developed and the medial vas deferens is shorter than in *C. confusus*, n. sp.

Habitat

Specimens recently collected were found in areas near natural vegetation but also near

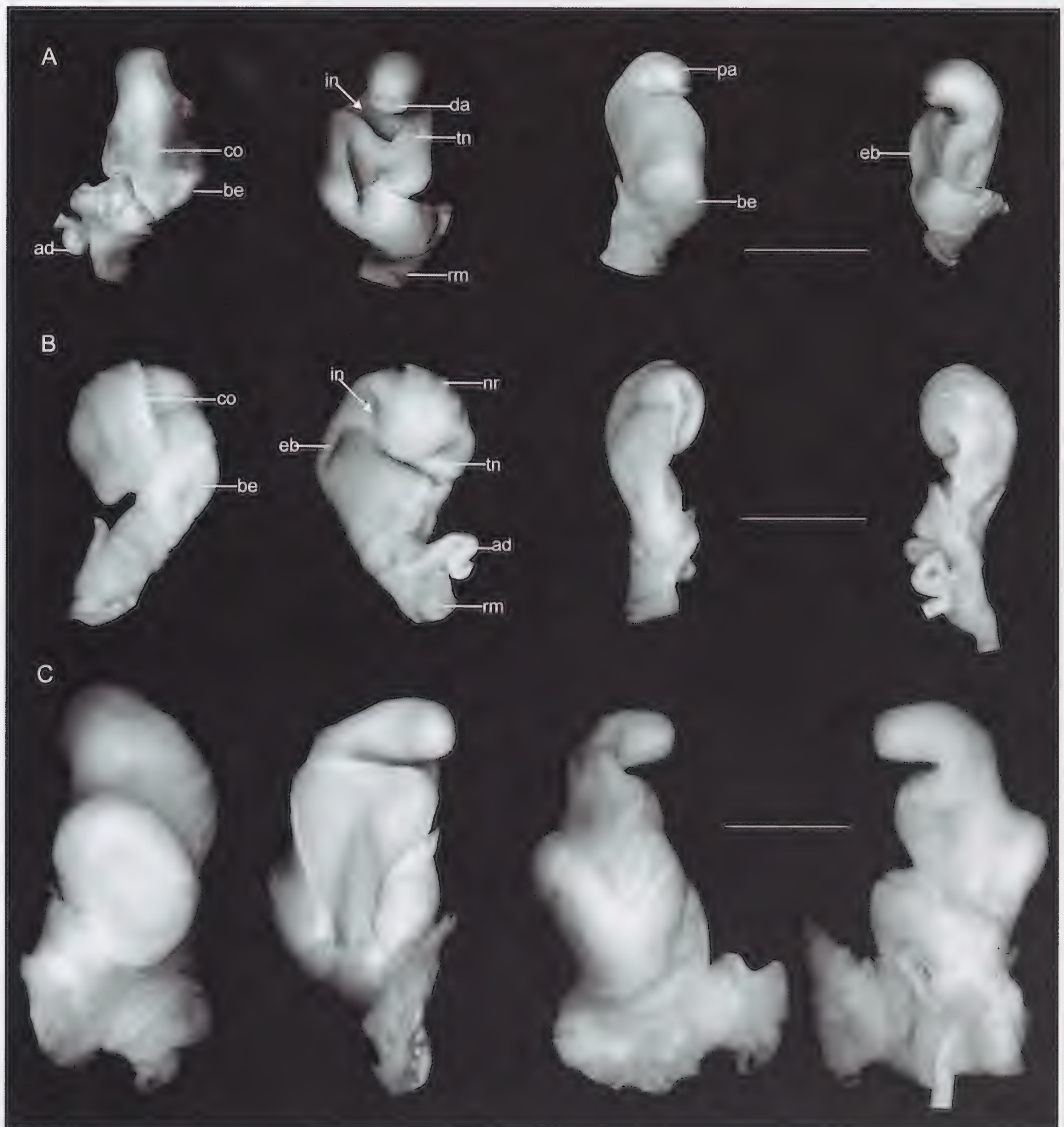


FIG. 12. Phallus of *Colosius propinquus* (A–B) and of *Colosius pulcher* (C) shown in the same proportion. A: Specimen from Reventador, Sucumbíos, Ecuador (QCAZ-M-003700); B: Specimen from El Angel, Imbabura, Ecuador (QCAZ-M-003659); C: Specimen intercepted by USDA in Miami from Ecuador in 1969 in Miami (USNM-769142). Ad - part of the anterior vas deferens penetrating in the phallus base; be - oval to rectangular swelling found on the basal region of the phallus; co - longitudinal and central swelling often visible on the phallus; da - location on the phallus where the vas deferens opens; eb - phallus expanded base that forms two expansions similar to lateral brims; In - shallow invagination delimited by a transversal ridge on the ventral side of the phallus, between its base and papilla; pa - phallus papilla; rm - phallus retractor muscle; tn - transversal ridge located between the base of the phallus and its papilla. Scale = 2.5 mm.

human habitation and in fields, as seen in the Volcán Pululahua in Paschoa, for example. During the day, they were found under rocks and woods. Most records of occurrence of *C. pulcher* are at high altitudes ranging from 1,229 to 3,400 m. Only two records are in low altitudes, which correspond to Santo Domingo (Pichincha) and Boliche (Cotopaxi), 479 m and 300 m above sea level, respectively.

Occurrence (Fig. 11)

Ecuador: Carchi Province: San Gabriel; Imbabura Province: Lita, Peguche, San Pablo; Pichincha Province: Aloag, Calderón, Volcán Pululahua, Pifo, Paschoa, Quito, Santo Domingo; Napo Province: Archidona, Borja, Oyacachi, Papallacta; Cotopaxi Province: Boliche, Iliniza Sur, Latacumba, Parque Nacional, Pastocalle, Pujilí; Chimborazo Province: Penipe; Cañar Province: Cañar (Colosi, 1921); Azuay Province: Cuenca, Gualaceo, Sigisig (Colosi, 1921).

Genetic Variability

Genetic variation within *C. pulcher* samples were measured using fragments of the 16S and COI mitochondrial genes. Based on small sample sizes (N = 5 for 16S and N = 8 for COI) genetic diversity was greater within *C. pulcher* than in *C. confusus*, n. sp. Although COI diversity was slightly greater in *C. pulcher* (0.3%) than in *C. confusus*, n. sp., (0.2%), diversity between two *C. pulcher* samples could be over 1% for 16S. This variation is still much lower than distances that separate species (i.e., > 4%). In the phylogenetic analysis *C. pulcher* was most closely related to *C. propinquus*. Although reddish and brown color-forms of this species have been found, our analysis of four brown and four reddish specimens using COI did not reveal evidence of genetic separation between color forms.

Remarks

There is only one record of *C. pulcher* intercepted by the USDA to date. This corresponds to a specimen examined by Thomé et al. (1997) and re-examined in this study, which had been intercepted on bromeliads from Ecuador on March 18th, 1969 (USNM 769142).

DISCUSSION

Only *C. lugubris* and *C. pulcher* were included with certainty in *Colosius* by Thomé (1975), while *C. propinquus*, *C. buergeri* and *C. festae* were included in the genus with reservations. The *C. propinquus* morphology is very similar to *C. pulcher*, but the phallus is less voluminous, as described by Colosi (1921, 1922) (Fig. 12). *Colosius propinquus* was considered an unidentified species by Thomé (1993) and a synonym of *C. pulcher* by Hoffmann (1925) and Kraus (1953). Two examined adult specimens from different localities (from El Angel, Imbabura; and from Reventador, both in Ecuador) near the type locality of *C. propinquus* (El Pun, Ecuador), match Colosi's morphological description (1921) for this species. Both specimens also showed the same haplotypes. Adult specimens of a form morphologically similar to *C. buergeri* (described originally based on juvenile specimens and which holotype is lost) were recently collected from Dominican Republic (Robinson et al., unpublished). This species, however, is related to species of Veronicellidae from Antilles, according to molecular data of a study in preparation by Robinson et al. Although the holotype of *C. festae* has been lost (Thomé, 1993), the phallus described by Colosi (1921, 1922) and Hoffmann (1925) provide sufficient information to distinguish it from *C. confusus*, n. sp., and from the other species of the genus. Although the monophyly of the genus is recovered in the trees, this relationship is not well supported by branch support values and is based on limited taxonomic sampling. Additional studies are required to formally test phylogenetic placements and monophyly of the genus.

Colosius confusus, n. sp., and *C. pulcher* can be externally indistinguishable, particularly when both have a dark brown notum (Figs. 2G, I, 8A). As in all species of the genus, these two species have a digitiform gland with very branched tubules at their base with a thick bursa copulatrix duct. Both species also have the digitiform gland with two kinds of tubules: internal short tubules and external long tubules, as seen in several other American genera (Thomé, 1975). Pedal gland, pedal and palial nerves, and the pedal artery are similar to several other American species of Veronicellidae (Thomé, 1975; Gomes & Thomé, 2004). In both species the phallus, considered the

principal diagnostic character in Veronicellidae, is leaf-like, with an expanded base that forms two lateral brims, and a conical papilla in their distal extremity. Base and papilla are delimited by a transversal ridge that borders a shallow invagination on the ventral side of the phallus (Figs. 3, 9, 12).

Despite sharing so many similarities, *C. confusus*, n. sp., and *C. pulcher* can be also differentiated by characteristics found on the phallus, digitiform gland and region of the bursa copulatrix. *Colosius confusus*, n. sp., has a deep longitudinal groove extending from the phallus base to its distal region, near the

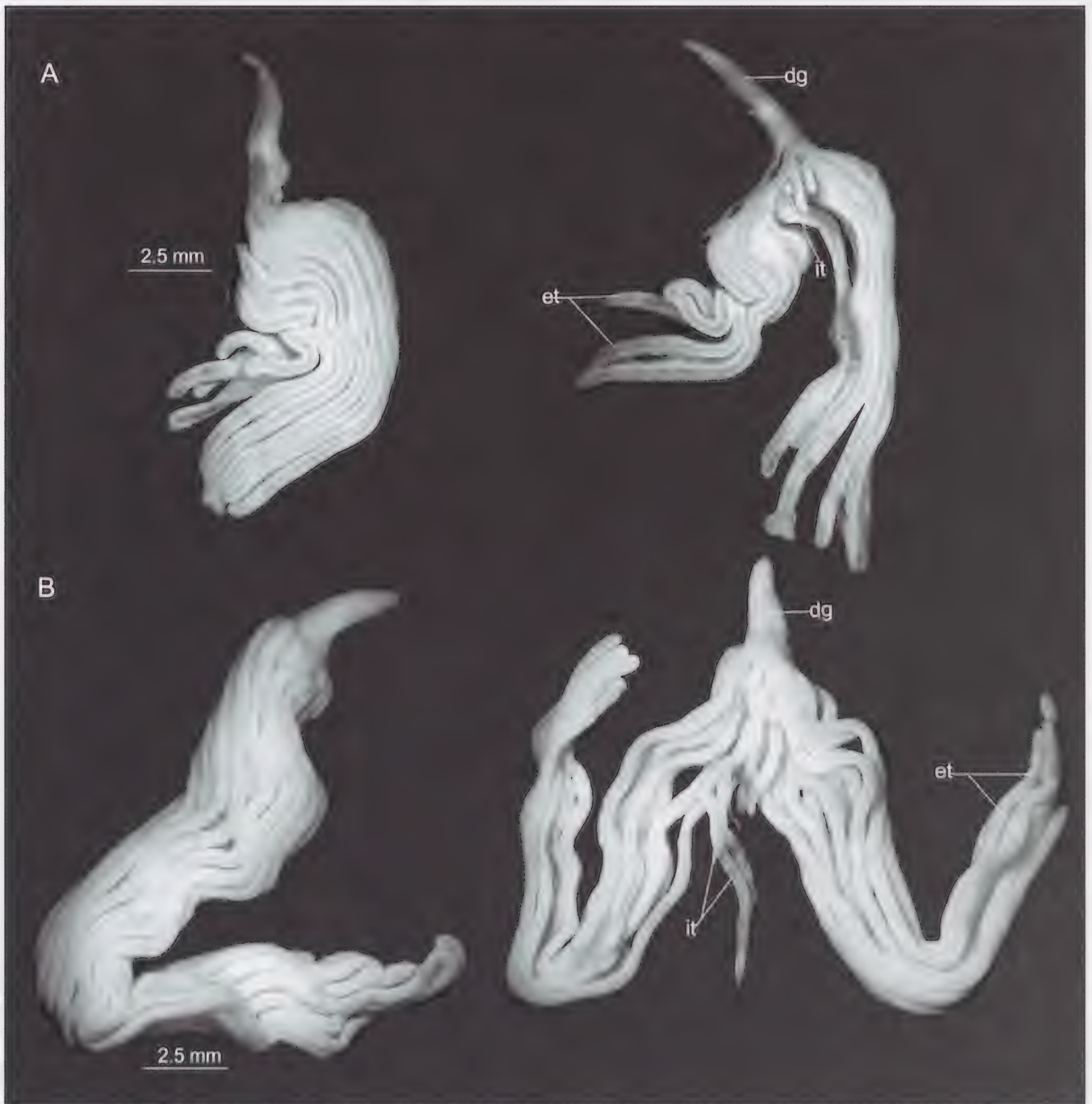


FIG. 13. Digitiform gland in a natural position and after the separation of the external tubules, to show the short internal tubules. A: Specimen of *Colosius confusus*, n. sp., in Miami on November 24th, 2007 (USDA-110451); B: *Colosius pulcher* from Volcán Pululahua, Pichincha, Ecuador (USDA-110423). Dg - papilla of the digitiform gland; et - long external tubules; it- short internal tubules.

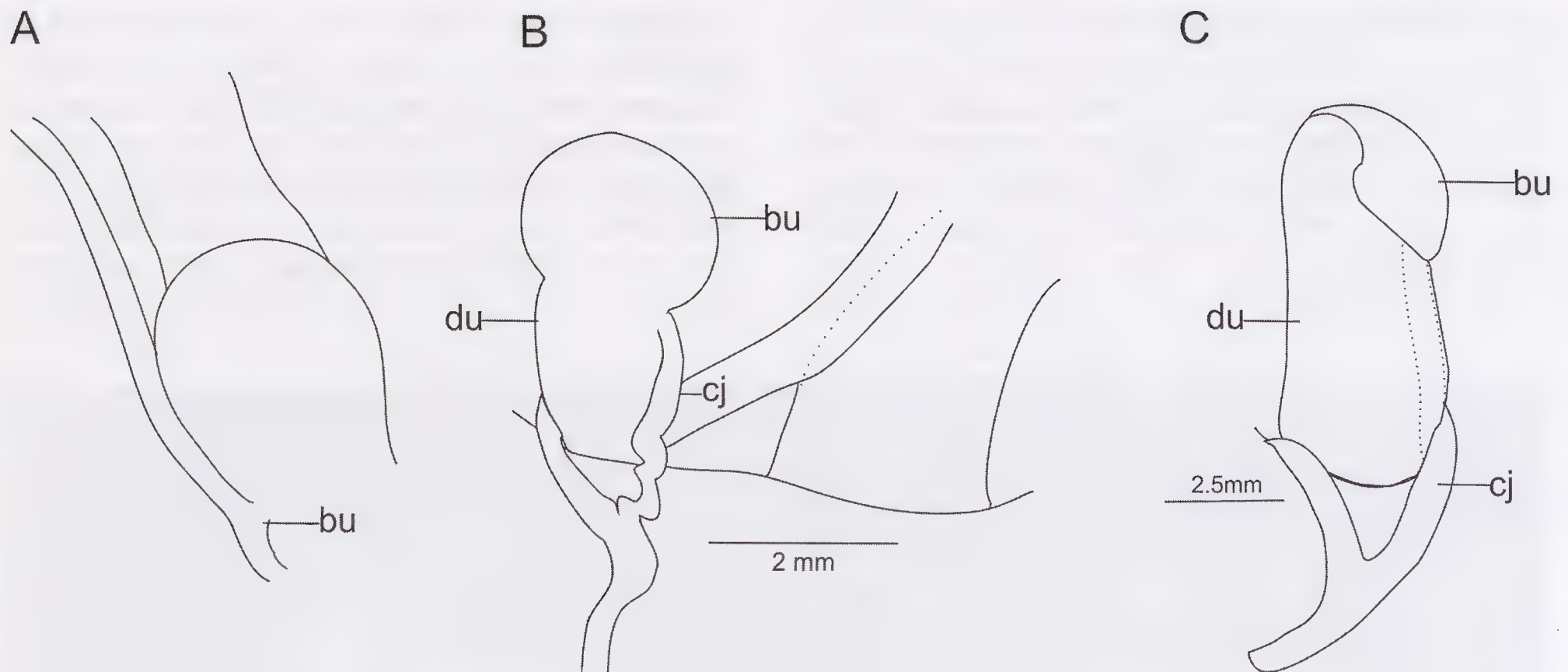


FIG. 14. Phallus illustration for *Colosius pulcher*. A: According to Colosi (1922); B: According to Thomé 1970b; C: The same position in ***Colosius confusus***, n. sp.

papilla. The dorsal side of the phallus is also covered by numerous fine raised threads. In *C. pulcher* the groove is absent and there is an oval to rectangular swelling at the basal region of the phallus (Figs. 3, 9). A few ridges are also often on the right lateral expansion of the phallus. In ***C. confusus***, n. sp., the internal short tubules range from 1 to 3 and in *C. pulcher* from 5 to 12 (Fig. 13). In juveniles these structures (groove and swelling) are also present on the phallus, although they are less developed. The number of tubules of the digitiform gland is also the same in young and adult specimens. The ridges on the phallus, however, are often absent in juvenile specimens. Both have a bursa copulatrix duct with a very thick duct with well-developed internal pilasters, but in ***C. confusus***, n. sp., the bursa duct is laterally expanded. The canalis junctor is also peculiarly long and sinuous in ***C. confusus***, n. sp. In addition, genetic differences in the DNA sequences of the species can be used to distinguish the species. Although not developed into a routine diagnostic method, the number of gaps and large divergence values between species provide useful information for confirming identification (Table 2). Genetic diversity within the 16S gene has been shown to be low within ***C. confusus***, n. sp., and *C. pulcher* and could serve as a practical diagnostic resource.

A leaf-like phallus with an expanded base and a distal papilla delimited from each other by a transversal ridge on the ventral side of the

phallus, where a shallow invagination can also be seen, so far described only for *C. pulcher* (and *C. propinquus*), additionally to the fact that *C. pulcher* was known only based on its original description and redescription of its lectotype and lectotypes, where only one similar phallus view was shown (Colosi, 1922; Hoffmann, 1925; Thomé, 1970b) (Fig. 14), as well the lack of studies about the Andean species of Veronicellidae were surely reasons that contributed to a series of mis-identifications involving *C. pulcher* (Thomé et al., 1997; Thomé et al., 2001; Constantino et al., 2010). Thomé et al. (1997) examined two specimens that he identified as *C. pulcher*, one from a USDA interception on bromeliads from Ecuador (USNM-769142) and another from the Dominican Republic (ANSP-A1092). The intercepted specimen is confirmed *C. pulcher* (Fig. 9B) and represents the only specimen intercepted to date, while the specimens from the Dominican Republic are a different species. This species is the same as the one recently found by Robinson et al. from the Dominican Republic (possibly an adult of *C. buergeri*). The species that Thomé et al. (2001) and Kraus (1953) called *C. pulcher* from Peru is an undescribed species, which will be described in a study in preparation, where the genus *Colosius* will be revised. The species that Constantino et al. (2010) called *C. pulcher* is ***C. confusus***, n. sp. (DPE CMS-1076), the new species proposed here. Thus, *C. pulcher* records, so far, are restricted to Ecuador (Fig. 11).

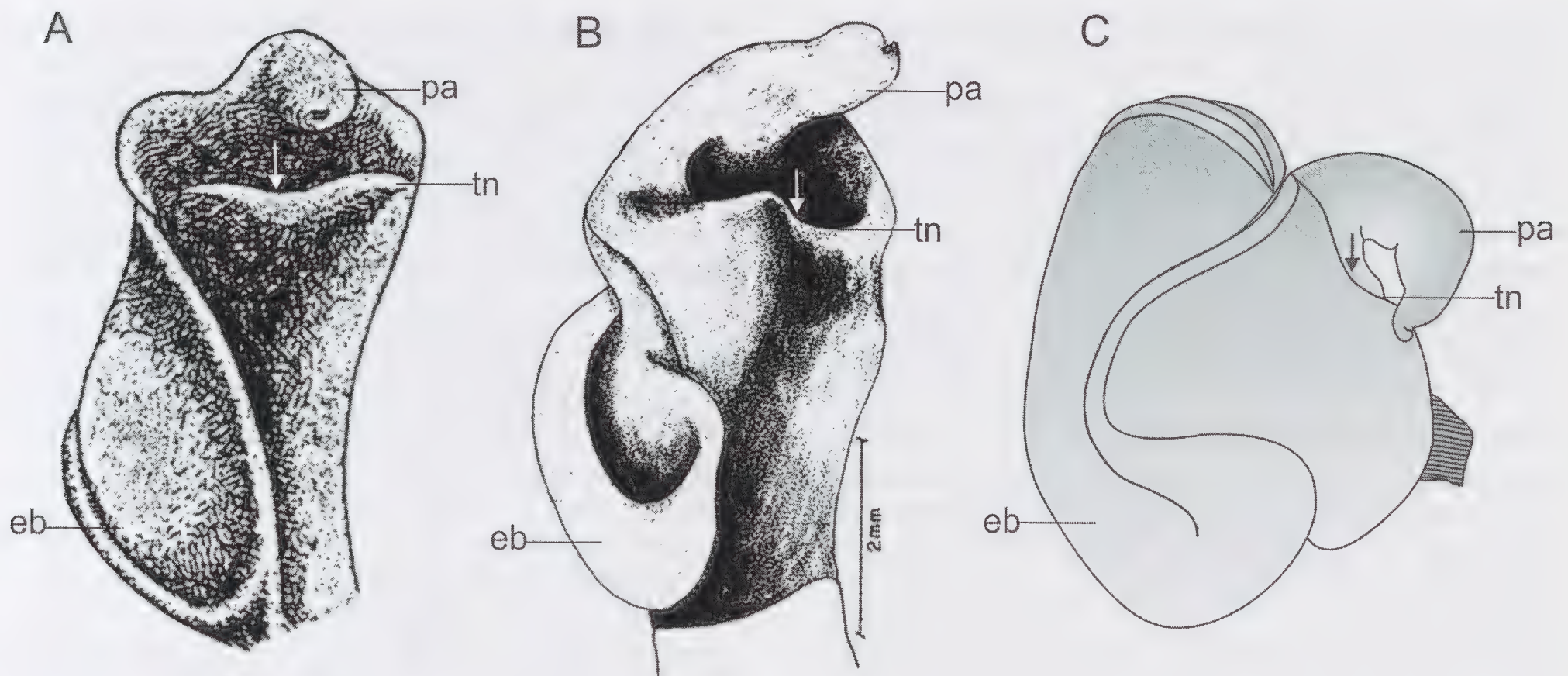


FIG. 15. Bursa copulatrix illustration for *Colosius pulcher*. A: According to Colosi (1922); B: According to Thomé (1970b); C: The same region in ***Colosius confusus***, n. sp.

Another issue involving *C. pulcher* is related to morphology of the bursa copulatrix. Colosi (1922) described it as being sessile with an extremely short duct where the canalis junctor penetrates. Thomé (1970b), based on specimens that he proposed as lectotype and paralectotypes, described the bursa copulatrix as formed by a very thick and well-developed duct, where the canalis junctor penetrates, near the bursa proper. According to our analysis, the bursa duct is well developed as described by Thomé (1970b) for the species, but it penetrates the bursa proper, instead of the bursa duct, different than that described for *C. pulcher* by these authors (Figs. 10, 15). An accessory gland is also present between the bursa duct and the rectum, in opposition of the description for this species held until now.

Colosius confusus, n. sp., was found in several locations in the Andean region of Ecuador in regions of flowers and coffee production in Colombia (Fig. 6). In Peru, it was found attacking coffee in Canchaque (northern Peru) and in two other Departments (Amazonas and Cajamarca). In Amazonas, it was found sympatrically with native species in urban areas, although not causing agricultural damage. The form found in Amazonas (2H, I) is also externally different from the forms found attacking coffee (Fig. 2A–G), although no genetic variation was found between them. The oldest specimen of the species in Peru was collected in 1970. In Ecuador, it was the second most commonly found species of Veronicellidae from

the Andean region, after *C. pulcher*, which is undoubtedly the most common one. Nevertheless, even after Colosi (1921, 1922) examined a large number of specimens from Ecuador, the author did not describe ***C. confusus***, n. sp. The first confirmed specimen of ***C. confusus***, n. sp., from Ecuador was collected in 1985 in Baeza, Napo province. Genetic information could aid in our understanding of ***C. confusus*** population structure, ancestral range, and dispersal patterns. The genes regions analyzed for our study, however, were selected to understand taxonomic differences. Although mitochondrial DNA (16S and COI) can provide information on the existence of isolated populations and evidence of migration, the diversity values measured within the species were low. This suggests that additional genes should be explored to study *Colosius* population genetics. The lack of variation observed in ***C. confusus***, n. sp., specimens from Ecuador, Colombia and Peru using mitochondrial DNA sequences is consistent with a recent migration/introduction between countries. Consequently, the large number of intercepted ***C. confusus***, n. sp., and material recently collected analyzed for 16S could not be traced back to a specific source population. ***Colosius confusus***, n. sp., was first intercepted by the USDA in 2001 on *Gypsophila* sp. from Ecuador. Only since 2003 has there been a significant increase of ***C. confusus***, n. sp.'s interceptions from Colombia (Fig. 7), mainly on *Hydrangea* sp., even though the United States has imported

aproximately 80% of Colombian flowers during the period 1997–2001 (USITC, 2003). The damage to coffee crops in Colombia seems secondary. Only recently, Constantino et al. (2010) reported the species (as *C. pulcher*) causing damage to coffee plantations in Colombia, in the municipality of Neira (Caldas), at 1,700 m altitude. A populational study based on a large number of specimens of *C. confusus*, n. sp., from a wide geographic area would be needed to determine its origin, but our results give some indications that the species is likely to be exotic to both Ecuador and Colombia, and may originally be from Peru. In Ecuador, the production of cut flowers began in the late 1970s with exports in the early 1980s (Acción Eco, 2000). The spread of *C. confusus*, n. sp., could be related to expanding commercial flower production.

The continuous and rapid increase of interceptions of *C. confusus*, n. sp., by agricultural inspectors in the United States supports the emergence of a increasingly important agricultural pest that is still uncontrolled, with a potential ability to adapt and become a pest of different kinds of crops, with a great potential of being spread due to its close association with the cut-flowers industry. It offers thus significant economic risk, which also poses an environmental risk including be competing with native species.

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